EFFECT OF DIURNAL CYCLING TEMPERATURES ON CARDIOVASCULAR-VENTILATORY FUNCTION IN STATICALLY-ACCLIMATED RAINBOW TROUT, *Salmo gairdneri*

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ABSTRACT

Four groups of rainbow trout, Salmo gairdneri, were acclimated to 2°, 10°, and 18°C, and to a diurnal temperature cycle (10° ± 4°C). To evaluate the influence of cycling temperatures in terms of an immediate as opposed to acclimatory response various ventilatory-cardiovascular rate functions were observed for trout, either acclimated to cycling temperatures or acclimated to constant temperatures and exposed to a diurnal temperature cycle for the first time (10° ± 4°C for trout acclimated to 10°C; 18° ± 4°C for trout acclimated to 18°C). Gill resistance and the cardiac to ventilatory rate ratio were then calculated. Following a post preparatory recovery period of 36 hr, measurements were made over a 48 hour period with the first 24 hours being at constant temperature in the case of statically-acclimated fish followed by 24 hours under cyclic temperature conditions.

Trout exhibited marked changes in oxygen consumption ($\dot{V}_{O_2}$) with temperature both between acclimation groups, and in response to the diurnal temperature cycle. This increase in oxygen uptake appears to have been achieved by adjustment of ventilatory and, to some extent, cardiovascular activity. Trout exhibited significant changes in ventilatory rate (VR), stroke volume (Vsv), and flow ($\dot{V}_G$) in response to temperature. Marked changes in cardiac rate were also observed. These findings are discussed in relation to their importance in convective oxygen transport via water and blood at the gills and tissues.

Trout also exhibited marked changes in pressure waveforms associated with the action of the respiratory pumps with temperature. Mean differential pressure increased with temperature as did gill resistance and utilization. This data is discussed in relation to its importance in diffusive oxygen transport and the conditions for gas exchange at the gills.

With one exception, rainbow trout were able to respond to changes in oxygen demand and availability associated with changes in temperature by means of adjustments in ventilation, and possibly perfusion, and the conditions for gas exchange at the gills. Trout acclimated to 18°C, however, and exposed to high cyclic temperatures, showed signs of the ventilatory and cardiovascular distress problems commonly associated with low circulating levels of oxygen in the blood. It appears these trout were unable to fully meet the oxygen requirements associated with cycling temperatures above 18°C. These findings were discussed in relation to possible limitations in the cardiovascular-ventilatory response at high temperatures.

The response of trout acclimated to cycling temperatures was generally similar to that for trout acclimated to constant temperatures and exposed to cycling temperatures for the first time. This result suggested that both groups of fish may have been acclimated to a similar thermal range, regardless of the acclimation regime employed. Such a phenomenon would allow trout of either acclimation group to respond equally well to the imposed temperature cycle.

Rainbow trout showed no evidence of significant diurnal rhythm in any parameters observed at constant temperatures (2°, 10°, and 18° C), and under a 12/12 light-dark photoperiod regime. This was not taken to indicate an absence of circadian rhythms in these trout, but rather a deficiency in the recording methods used in the study.

ACKNOWLEDGEMENTS

I would like to thank Dr. A.H. Houston for his guidance and encouragement throughout this investigation.

I would also like to express my appreciation to the following people: Ms. K.M. Mearow, Mr. L.S. McCarty and Mr. J.S. Smeda for their numerous contributions throughout the study, the staff of the Technical Services Department, for the construction and maintenance of laboratory equipment, and Mrs. R. French for typing the document.

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INTRODUCTION

Few north temperate fishes occupy thermostable environments. Virtually all face major seasonal changes in temperature and many, notably those occupying shallow, slow-flowing habitats, must also adapt to substantial diurnal variations as well. Furthermore, heated discharges from industrial and municipal activities have in recent years lead to the increasing alteration of what might be termed 'normal' thermal circumstances. Such effects are particularly critical in the case of the cold water habitats occupied by commercially desirable species, including the salmonid game fishes. Seasonal baseline temperatures may be altered in such localities, discharge patterns may affect diurnal temperature variations and episodically indigenous species may be transiently exposed to lethal or near-lethal increases in temperature.

Increases in temperature impose a variety of stresses upon aquatic organisms. Paramount among these is the necessity of satisfying enhanced oxygen requirements under circumstances in which oxygen content, and frequently oxygen tension have been significantly reduced. Omitting possible adjustments in intermediary metabolism which may be used to reduce thermal effects on metabolic activity, the teleost can, in essence, respond along two general lines to meet this challenge. It can alter the functional properties of the cardiovascular-ventilatory-branchial complex responsible for transfer of oxygen from the respiratory medium to the blood. Alternatively, or in conjunction with this, it can adjust overall blood oxygen-carrying capacity and (or) hemoglobin-oxygen affinity, and thus the amount and availability of oxygen to tissues.

The latter aspect of response has been the subject of several studies. In the rainbow trout, the subject of the present study, acclimation to higher temperatures is associated with some increase in total hemoglobin

content and erythrocyte numbers, reduction in mean red cell volume and minor changes in mean erythrocytic hemoglobin content (DeWilde & Houston, 1967). Both the relative and absolute abundancies of several components of the hemoglobin system are significantly altered during the acclimatory process (Houston & Cyr, 1974). Red cell levels of ATP and GTP, the principal organophosphate modulators of hemoglobin-oxygen affinity in this species are not influenced by temperature (Weber, Wood, & Lomholt, 1976). However, the exothermic nature of hemoglobin-oxygen interaction, and the sensitivity of several of the trout hemoglobins to the reductions in pH which inevitably accompany increases in temperature would be expected to reduce hemoglobin-oxygen affinity and facilitate oxygen unloading at the tissue level (Brunori, 1975). These factors, plus changes in ionic modulator concentrations (Houston & Smeda, 1976) presumably account for the modest decreases in affinity reported by Weber et al. (1976).

Response at the systemic level has not as yet received comparable attention despite long-standing knowledge of the magnitude of thermal effects upon oxygen demand in rainbow trout (Beamish, 1964; Brett, 1964; Fry, 1967). The absence of such information is surprising in view of the increasing thermal pollution problems alluded to initially, and the existence of detailed analyses of systemic responses to hypoxia (Holeton & Randall, 1967 a & b) and to exercise-induced increases in oxygen consumption (Stevens & Randall, 1967 a & b). A primary goal of the present study has therefore been analysis of ventilatory, and to a lesser extent cardiovascular responses, in terms of temperature-related parameters such as: oxygen consumption, percent utilization, ventilatory flow, ventilatory rate, heart rate.....etc.

A second major aim has been that of examining the influence of diurnally-

cycling, as compared to constant temperature conditions upon the activities of the exchanger complex. Virtually all studies upon thermoacclimatory phenomena have been conducted by reference to constant temperature. Little is known as yet with respect to response to the ecologically more realistic circumstance of diurnal temperature fluctuation. Consequently, in the present study emphasis has been given to animals acclimated to 2°, 10°, and $18^{\circ}C$, and to specimens exposed to a diurnal, sinusoidal cycle of $10 \pm 4^{\circ}C$. In addition, in an attempt to evaluate immediate, as opposed to acclimatory responses to cycling temperatures animals acclimated to and tested under constant temperature conditions were exposed to one or more cycles upon completion of initial recordings under constant conditions.

Literature Review

As has been indicated in the Introduction increases in oxygen uptake at higher temperatures place a considerable demand upon the cardiovascular-respiratory capabilities of teleost fish. The following Literature Review has been divided into sections which serve, first, to describe the important components of the branchial exchanger complex, and second, to identify potentially modifiable components which might be adaptively adjusted to enhance overall oxygen transport capability. In these respects particular emphasis has been given to:

1. gill structure and function
2. the ventilatory system
3. gill resistance
4. the circulatory system
5. analysis of gas exchange
6. the effect of temperature

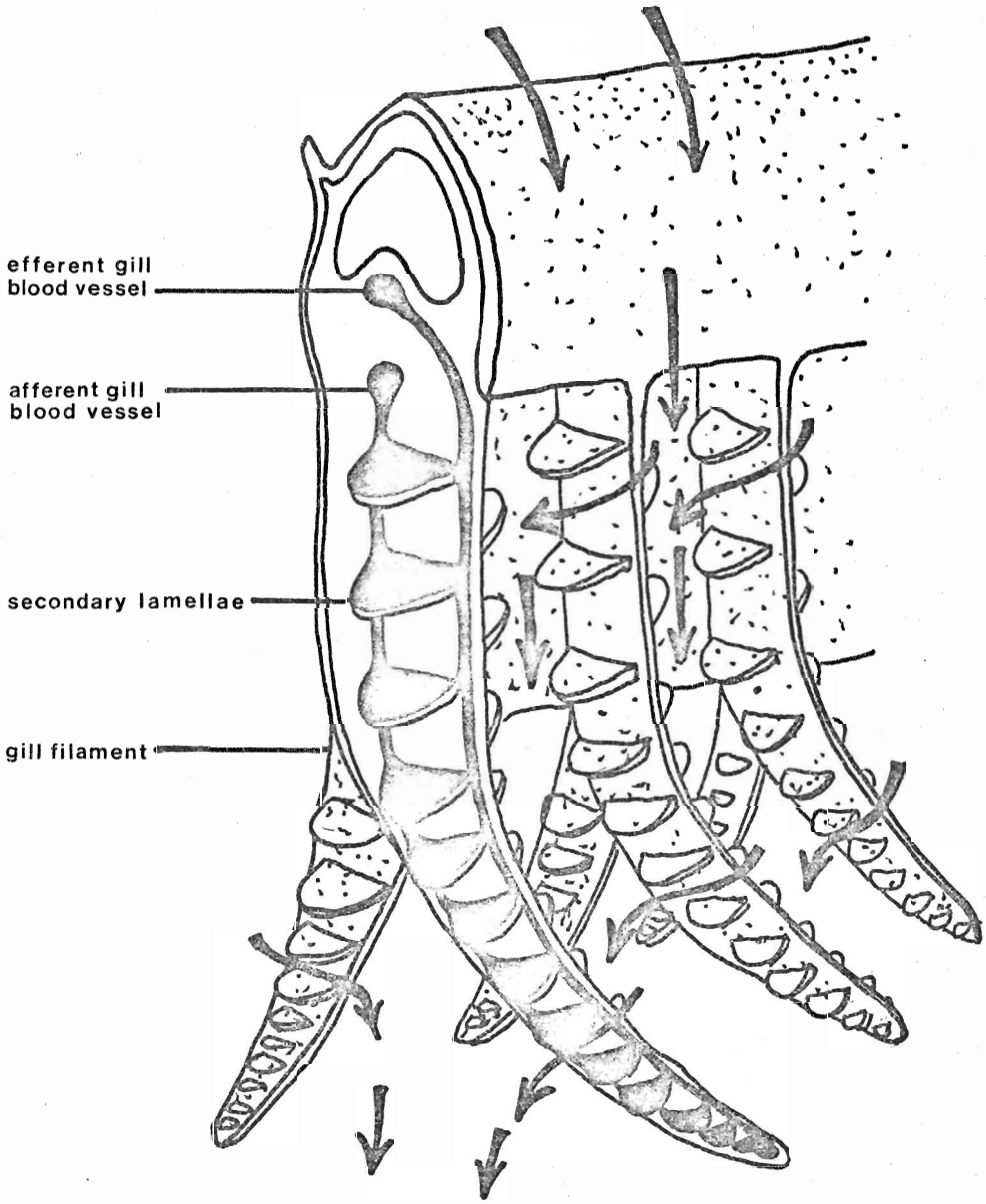
1) Gill Structure and Function.

The gross morphology of the fish gill has been described by several authors (Hughes, 1961, 1966, 1970; Steen & Kruysse, 1964; Hughes & Morgan, 1973). The ventilatory system consists of buccal and opercular cavities functionally separated by the gills. Their structure is such that the transverse secondary folds (secondary lamellae) of the primary filaments interdigitate, forming a continuous sieve. Water is pumped over the gills through the small channels between the secondary lamellae and at right angles to the gill filaments (Fig. 1).

The secondary lamellae are composed of an internal layer of pillar cells which form the blood space, and are surrounded by basement membrane and an external layer of epithelial cells (Hughes & Morgan, 1973). Recently, tertiary folds or microvillae have been found on the outer surface of the epithelium (Hughes, 1970). As well as increasing overall surface area these folds or microvillae may be involved in anchoring the mucous film which normally covers the entire secondary lamella. Proposed functions of this mucous layer include; (1) protection of the underlying epithelium from foreign bodies and bacterial infection, (2) reductions in the drag encountered by fast-swimming species, (3) contributions to overall differential permeability of the gill epithelium to specific ions and to water and the respiratory gases, O_2 and CO_2. The latter possibility would confer important adaptive advantages on fish living in fresh water, for under these conditions large gill areas result in ionoregulatory and osmoregulatory problems upon exposure to high temperatures (Hughes, 1970; Johansen, 1971; Randall, 1970b; Hughes & Morgan, 1973).

In terms of the mass transfer of respiratory gases, two important gill parameters are total surface area for gas exchange, and the water-to-blood diffusion distance. Quantitative aspects of gill morphology have

Figure 1. Diagram of a portion of the gill arch of a teleost fish. (modified from Hughes and Morgan, 1973).


efferent gill blood vessel
afferent gill blood vessel
secondary lamellae
gill filament
direction of water flow

been reviewed by various authors (Hughes, 1970; Randall, 1970b; Hughes & Morgan, 1973). The most immediate feature of such studies is that although large quantitative variations exist, gill structure is closely related to weight, activity, and habitat conditions (e.g., O_2 availability, pH, temperature, etc.). Several general trends can be identified, however, and include the following.

1) Total gill area per unit body weight is exponentially related to weight by an exponent ranging from 0.85 to 0.90 (Muir & Hughes, 1969; Hughes & Morgan, 1970; Johansen, 1971). Consequently, within any given species therefore, smaller fish have larger weight-specific gill areas than do larger specimens.

2) The area, frequency, spacing and height of secondary lamellae varies along the length of the filament within individual fish.

3) More active fish (pelagic) such as mackerel, possess large numbers of closely spaced, short secondary lamellae. Sluggish species are characterized by secondary lamellae which are higher and more widely spaced (Hughes, 1966).

4) In very active fish (tunny) total gill area is greater than in less active fish. This is achieved by an increase in filament length. Consequently, increases in the number of secondary lamellae can be achieved without substantial decreases in spacing which would greatly increase resistance to flow. More sluggish fish tend to have shorter filaments with higher and more widely spaced lamellae. Thus, less active fish have a coarser gill sieve which offers less resistance to water flow relative to the total area than do more active forms (Hughes, 1966).

5) The effective exchange area (consisting of the area of the blood channels in the lamellae) represents only 60 to 70% of the anatomical surface gill area (Hughes, 1966). The remaining surface of the secondary lamellae

lies above the ends of the pillar cells and is not able to take part in gaseous exchange. Randall (1970b) has shown that the former value represents the maximum functional exchange surface area. In the resting rainbow trout the effective surface area may be as little as 20% of the anatomical area reported by Hughes (1966). The discrepancies in these two values point to the possibility that changes in effective exchange area can be achieved via perfusion adjustments. Such a mechanism would confer an important adaptive advantage to the fish under conditions of increased oxygen demand like that associated with high temperatures.

6) The water-to-blood barrier to oxygen and carbon dioxide diffusion consists of the epithelium, basement membrane, and pillar cell flange. As with gill area this parameter differs interspecifically, and is related to body weight and activity to some degree. Within any species, larger specimens tend to have longer distances (Hughes & Morgan, 1973), while more active fish with larger gill areas tend to have the shortest distances (Hughes, 1970). It has been suggested (Johansen, 1971: Hughes & Morgan,1973) that water-to-blood distance may also be related to habitat, with species from hypoxic and fresh water environments having shorter distances than fish from normoxic and marine environments.

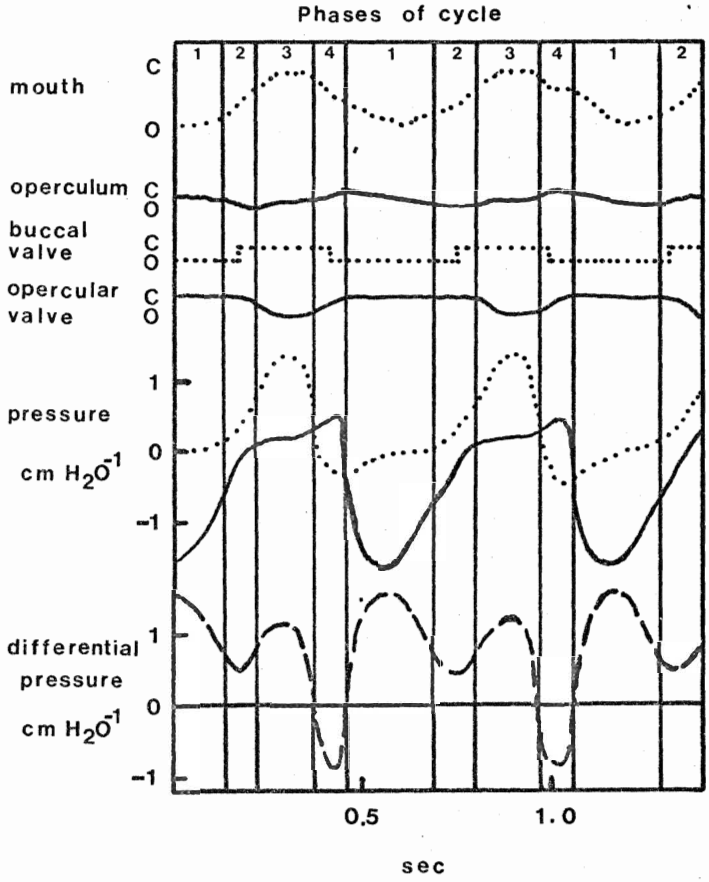
In summary, the quantitative analysis of gill morphology has served to illustrate the complex integration of one aspect of the respiratory system with respect to some general factors such as activity, weight, and habitat. In addition, this general survey suggests that selection has operated on various aspects of the branchial system to maximize efficiency under varying environmental conditions.

(b) The Ventilatory System

Gill ventilation in fishes has been described by several authors (Hughes & Shelton, 1958; Hughes, 1961, 1970; Saunders, 1961; Hughes & Roberts, 1970; Shelton, 1970) and is discussed in a number of reviews (Randall, 1970b; Johansen, 1971). The ventilatory system includes a double pumping mechanism, with the gill sieve, which represents the principal resistance to flow, situated between a positive pressure buccal force pump and a negative pressure opercular suction pump. The mechanics of ventilation as well as flow patterns and gill resistance has been studied in detail by Hughes and Shelton (1957, 1958), Saunders (1961), Ballintijn (1972), and thoroughly reviewed by Hughes and Morgan (1973). Manometric studies (Hughes & Shelton, 1958) have shown that water probably flows continuously across the gills. Pressure amplitudes from the buccal and opercular chambers indicate that with the exception of a brief reversal period, buccal pressure exceeds that in the opercular chamber throughout the respiratory cycle (Fig. 2). The period of differential pressure reversal is so brief, and the pressure difference so small, that an actual reversal of water flow was thought by Hughes (1961) to be unlikely due to inertial effects.

Ventilatory flow depends primarily upon the relationship between buccal-opercular pressure gradient and gill resistance. The exsitence of a semi-constant differential pressure (1 to 2 cm H_2O) suggests that the gill offers significant resistance to flow. It appears, however, that gill resistance and therefore water flow, varies during each cycle (Hughes, 1961). With respect to the latter, movements of the gill filaments have been observed through the use of cinĕ films (Hughes & Shelton, 1957, 1958; Hughes, 1961). Through the use of electromyographic techniques (Shelton, 1970; Ballintijn, 1972; Hughes and Morgan, 1973) the activity patterns of ventilatory muscles have been illucidated.

Figure 2. The breathing movements of the mouth and operculum, together with associated pressure changes in the buccal and opercular cavities. The differential pressure between these cavities is shown below. O and C indicate the open and closed position of the mouth, operculum and associated valves. For rainbow trout (70 gm) (modified from Hughes and Shelton, 1962).


Phases of cycle
1
2
3
4
1
2
3
4
1
2
mouth
C
O
operculum
C
O
buccal valve
C
O
opercular valve
C
O
pressure
cm H_2O^{-1}
1
0
-1
differential pressure
cm H_2O^{-1}
1
0
-1
0.5
1.0
sec

From these investigations it was concluded that the abductor and adductor muscles of the primary filaments function to expand and contract the gill sieve in relation to both pressure and volume changes in the buccal and opercular chambers. It is felt that by adjusting gill filament position the cost of ventilation is minimized through adjustments in resistance which maximize ventilation of the respiratory surfaces during all phases of the ventilatory cycle.

In a sense the muscles associated with the buccal and opercular pumps exhibit comparable variations during each cycle. A strong mechanical coupling between the various components of the pumping mechanism insures hydrodynamic efficiency in the face of increased ventilatory intensity. Ballintijn (1972) suggested that for any given steady state level of ventilation a specified number of muscles are active and working near their optimum performance levels. He proposed that the transition to higher levels of ventilatory intensity involved recruitment of a larger number of active muscles, and that each additional muscle brought into play further improved working efficiency (Ballintijn 1969, 1972; Ballintijn & Hughes, 1965).

Ventilatory control is not well understood in fish. The possible role of proprioception in regulating ventilation has been considered (see Johansen, 1971; Ballintyn, 1972 for references). Ballintijn (1972) suggested that proprioceptors on the gills, and in ventilatory muscles provide feedback information which is used to integrate water flow and gill resistance with changing ventilatory demands. This system is felt to regulate arterial blood oxygen levels during exercise. Crawshaw (1976, 1977) has proposed that an analagous system exists in fish which regulates blood oxygen levels during thermally induced increases in metabolic rate. In this case neural input from thermal receptors in the periphery and anterior brainstem to respiratory control centres in the central nervous system results in anticipatory increases in ventilatory activity with temperature.

(c) Gill Resistance

Early investigations into the mechanism of ventilation (Hughes & Shelton, 1948; Hughes, 1961, 1966) emphasized the significant resistance to water flow imposed by the gill, and it is clear that the relationship between flow velocity and the pressures generated in the buccal and opercular cavities is a function of this resistance. The role of gill resistance in regulating gas exchange has been reviewed by Randall (1970b) and Hughes and Morgan (1973). The dimensions of the gills are such (< 1 mm between adjacent lamellae) that it was initially questionable whether the differential pressures recorded during the normal ventilatory cycle (~1 cm H_2O) would be sufficient to maintain the large ventilatory flows observed (30 to 100 mls min^{-1}) (Hughes, 1966). Calculations based on a square tube model analogous to a gill sieve of the same dimensions gave fairly good agreement with the actual volumes of water being pumped over the gills however.

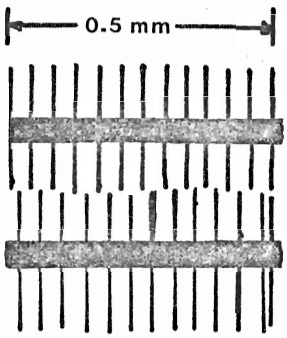
The importance of gill resistance in gas exchange stems from its relationship to ventilation volume* and percent utilization**. These two parameters will strongly influence the amount of oxygen which is removed from the water by the gills. With regard to ventilation volume, it was shown (Hughes, 1966) that although the total resistance of the gill sieve is large, the resistance of each pore is relatively small. In terms of utilization, the small pore size serves to reduce the maximum or physiological diffusion distance, i.e., that from the centre of the interlamellar space to the blood channel between the pillar cells.

Although direct estimates of gill resistance are not available, a qualitative description of its components is possible (Fig. 3c). The two main pathways for water flow through the gills are : (1) the axial pathway between the tips of adjacent filaments, and (2) the interlamellar pathway

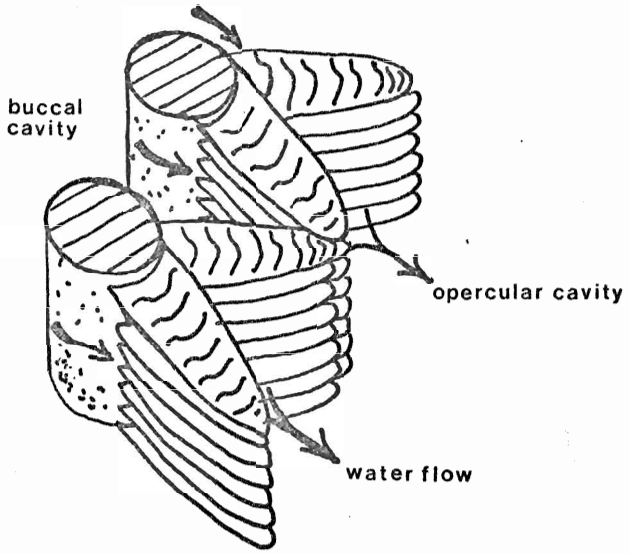
*volume of water pumped over the gills per unit time.

**amount of oxygen removed from water passing over the gills

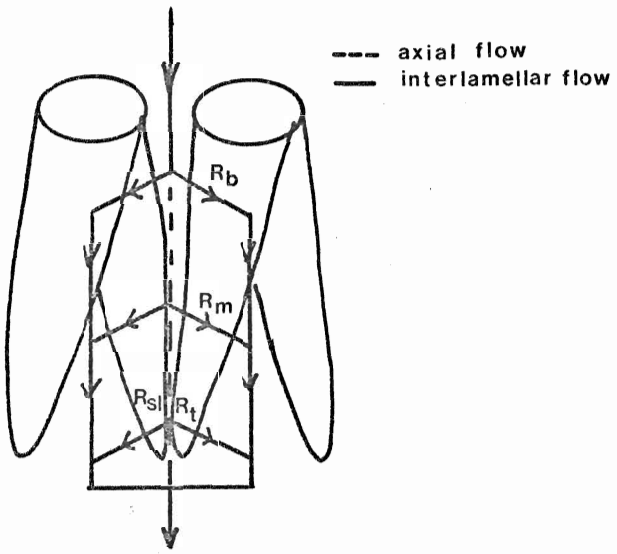
Figure 3. (a) Diagram of a portion of the gill sieve provided by the primary filaments and secondary lamellae of a tench. (b) diagram of two gill arches and the double row of primary filaments attached to them in a teleost fish. (c) diagram of two gill arches of a teleost fish showing the various components of the total resistance to the flow of water through the sieve. (a) and (b) are modified from Hughes, 1961; (c) modified from Hughes, 1972a).


0.5 mm

A


buccal cavity
opercular cavity
water flow

B


axial flow
interlamellar flow
R_b
R_m
R_{sl}
R_t

C

between the secondary lamellae. Each of these principal pathways includes a number of resistance components. The axial resistance is the sum of the resistances due to the gill arches and rakers (R_a), the gill filaments (R_f), and the slit (R_{sl}) where the filament tips are approximated. The interlamellar resistance consists of the component resistances of secondary lamellae; those at the base (R_b), in the middle section of the primary filament (R_m), and at the tip of each filament (R_t). Resistance will, of course, decrease toward the tip of the filament due to variations in the spacing and dimensions of secondary lamellae along the primary filament referred to earlier (pg. 12).

Hughes and Morgan (1973) proposed that during quiet ventilation overall resistance through the axial pathway and the tips of the filaments is relatively large due to the close juxtaposition of adjacent hemibranchs. Consequently, the main flow of water appeared to be through the basal and middle portions of the interlamellar pathway under such conditions.

Indirect estimates of gill resistance have been made by dividing mean differential pressure across the gills by the mean flow of water through the gills. Changes in this ratio are assumed to reflect changes in gill resistance. In trout (Hughes & Saunders, 1970) and tench (Hughes & Shelton, 1958) gill resistance changes with variations in mean differential pressure and ventilation volume, and appears to decrease as ventilation volume increases. Large increases in ventilation volume are also often associated with reductions in utilization (Hughes & Shelton, 1962; Saunders, 1962; Hughes, 1966; Hughes & Morgan, 1973). This suggests that the fall in total resistance may be a consequence of decreases in the component resistances associated with non-respiratory pathways (e.g., axial pathway) (Hughes, 1966). As ventilation volume increases the primary filaments are forced apart, decreasing the slit

resistance, and water then flows between the primary filaments rather than through the interlamellar sieve spaces, with a resulting drop in exposure to the exchange surface.

Further experiments (Hughes & Shelton, 1962; Hughes & Umerzawa, 1968) have, however, indicated that the relationship between gill resistance and flow cannot always be predicted by the ratio given above. At very high ventilation volumes, other features of the branchial system, including the size of the buccal and opercular openings limit ventilation (Shelton, 1970). It is also apparent that gill resistance varies during different phases of the ventilatory cycle due to (1) fluctuations in the mean differential pressure, and (2) movements of the branchial arches, gill filaments, and lamellae during the ventilatory cycle (Hughes & Shelton, 1962).

The model presented above, although speculative at best, gives some indication of how gill resistance will vary with changes in ventilation volume and differential pressure. In addition, resistance will vary between species as a function of gill morphology. In sluggish species with more widely spaced secondary lamellae total gill resistance is reduced more than is the case in active species (Hughes & Morgan, 1973).

Slit resistance, however, is relatively greater in sluggish species than in active fish because of the more complete interdigitation of the tips of the primary filaments. This, combined with the reduced interlamellar resistance in sluggish species, insures that a relatively large portion of the ventilated water flows over the actual gas exchange area. The low metabolic levels and oxygen requirements of sluggish fish thus allow them to meet their oxygen need under normal circumstances with ventilation volumes which do not increase water flow via axial nonrespiratory pathways. The problem with this type of strategy is that the geometry of the gills is such that gill resistance cannot then be increased sufficiently to maintain

the integrity of the sieve under conditions of high ventilatory demand. As a result, the efficiency of the branchial exchanger decreases sharply with increasing flow when the conditions for gas exchange are nonideal (e.g., hypoxia).

(d) The Circulatory System

(i) General considerations

The following section will serve to briefly review general hemodynamic relationships in the branchial and systemic components of the teleostean circulatory system. Literature pertinent to cardiovascular function in fishes has been reviewed several times, and recently by Randall (1968, 1970a), Johansen (1961), and Jones and Randall (1978).

The major circulatory pathways of teleosts are shown schematically in Figure 4, and semi-diagramatically in Figure 5. The branchial and systemic capillary networks are arranged in series, with the gills representing the first major resistance to blood flow. The main propulsive organ, the heart, consists of four chambers arranged in series (Randall, 1968, 1970). These are shown diagramatically in Figure 6. All chambers, with the exception of the bulbous arteriosus, are contractile. The bulbous, however, is elastic, and noncontractile. Unidirectional blood flow is maintained, in part, by simultaneous function of the heart as a ventricular force pump, and atrial suction pump, and in part, by the coordinated opening and closing of cardiac valves with myocardial contractions (Johansen, 1971). The coordinated interaction of the atrial and ventricular chambers also serves to increase the overall efficiency of the heart pump.

Reported valves for cardiac output in teleosts vary from 5 to 100 ml. kg^{-1}. min^{-1}, but generally fall within a much more narrow range; 15 to 30 mls. kg^{-1}. min^{-1} (Randall, 1970a). Blood volume has been estimated as approximately 5% of total body weight (Randall, 1970a). Circulation times, calculated by dividing blood volume by cardiac output, are relatively slow; being in the order of 2 to 5 minutes (Randall, 1970a).

The relationship between blood flow in the ventral aorta and pulse pressures recorded in the ventricle and bulbous arteriosus are given in

Figure 4. A schematic illustration of the major circulatory pathways of the cardiovascular system in salmon (modified from Smith and Bell, 1976).

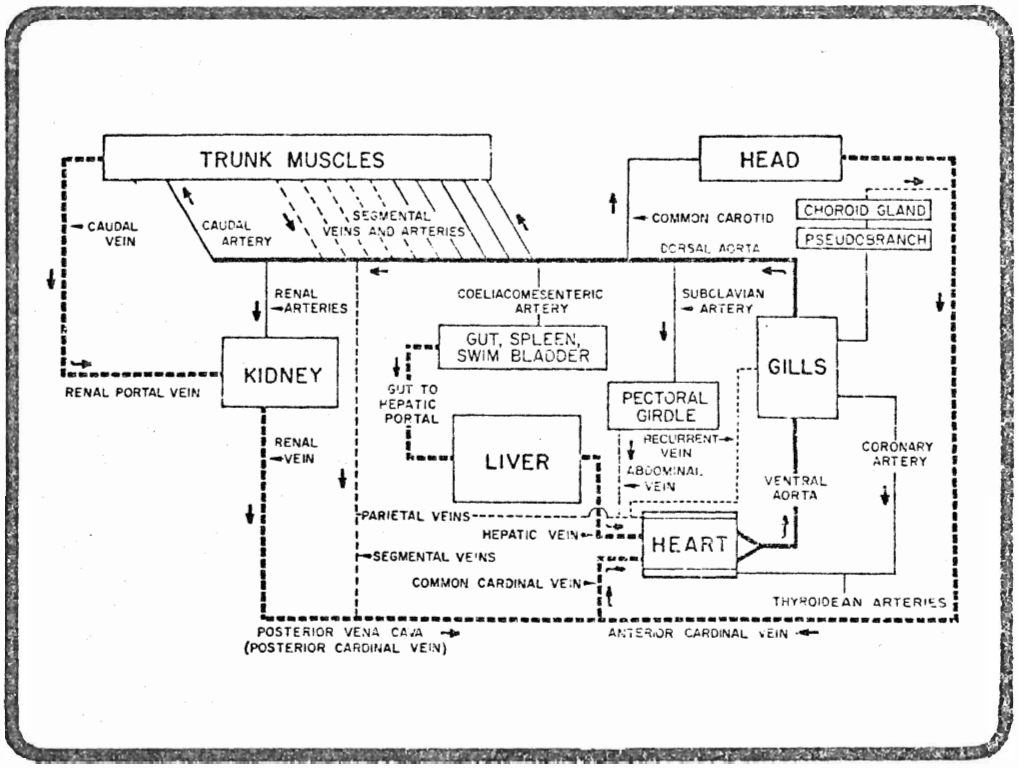

TRUNK MUSCLES
HEAD
CAUDAL VEIN
CAUDAL ARTERY
SEGMENTAL VEINS AND ARTERIES
COMMON CAROTID
CHOROID GLAND
PSEUDOBRANCH
DORSAL AORTA
RENAL ARTERIES
COELIACOMESENTERIC ARTERY
SUBCLAVIAN ARTERY
GUT, SPLEEN, SWIM BLADDER
KIDNEY
GILLS
RENAL PORTAL VEIN
GUT TO HEPATIC PORTAL
PECTORAL GIRDLE
RECURRENT VEIN
ABDOMINAL VEIN
RENAL VEIN
LIVER
CORONARY ARTERY
VENTRAL AORTA
PARIETAL VEINS
HEPATIC VEIN
HEART
SEGMENTAL VEINS
COMMON CARDINAL VEIN
THYROIDEAN ARTERIES
POSTERIOR VENA CAVA (POSTERIOR CARDINAL VEIN)
ANTERIOR CARDINAL VEIN

Figure 5. Semi-diagramatic representatation of the cardiovascular system of salmon (modified from Smith and Bell, 1976).

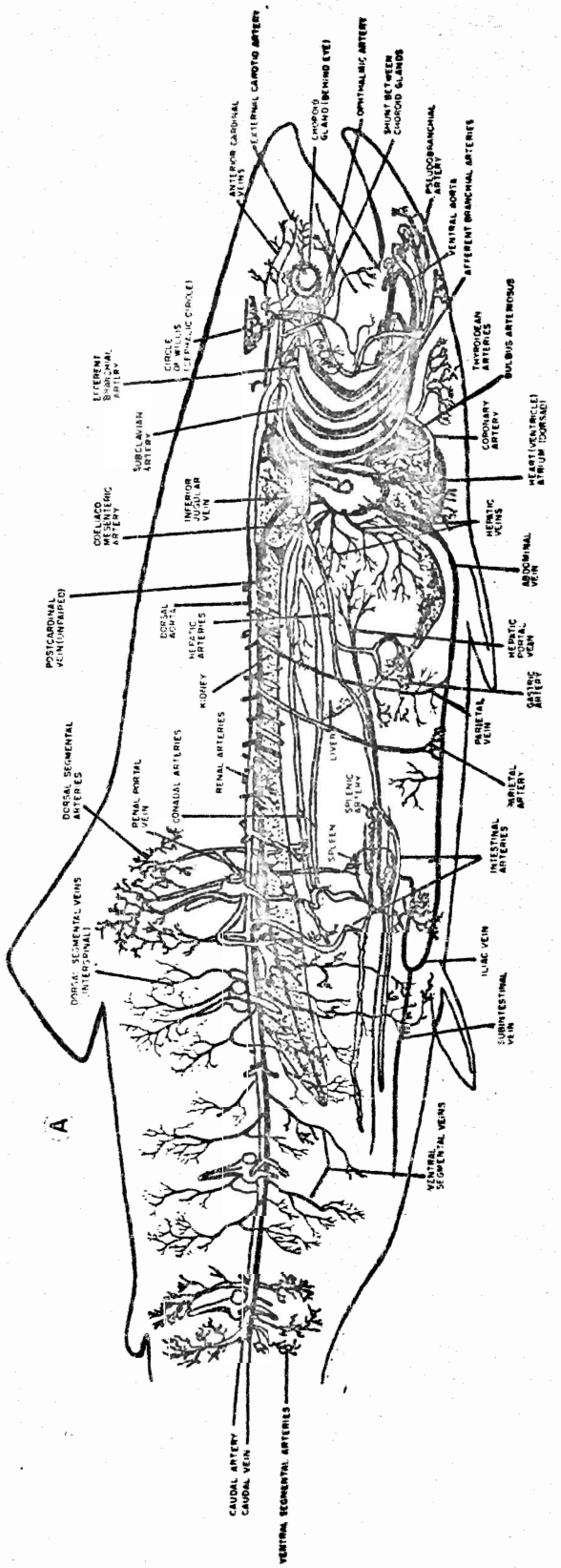
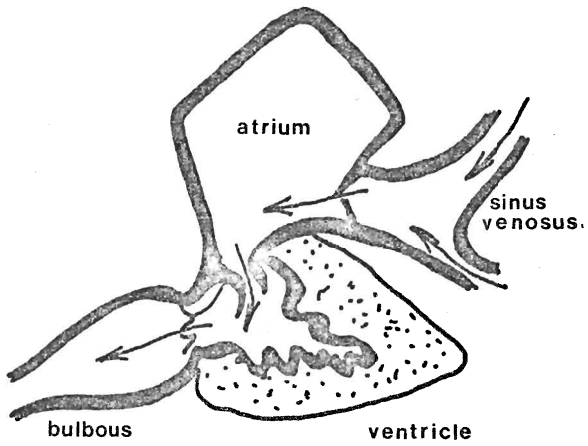

A
ANTERIOR CARDINAL VEINS
EFFERENT BRANCHIAL ARTERY
CIRCLE OF WILLIS (CEPHALIC CIRCLE)
SUBCLAVIAN ARTERY
CELIACO MESENTERIC ARTERY
INFERIOR JUGULAR VEIN
POSTCARDINAL VEIN (UNPAIRED)
DORSAL AORTA
HEPATIC ARTERIES
KIDNEY
RENAL ARTERIES
DORSAL SEGMENTAL ARTERIES
RENAL PORTAL VEIN
COMMISSURAL ARTERIES
LIVER
SPLENIC ARTERY
SPLEEN
INTESTINAL ARTERIES
ILIAC VEIN
SUBINTESTINAL VEIN
VENTRAL SEGMENTAL VEINS
CAUDAL ARTERY
CAUDAL VEIN
VENTRAL SEGMENTAL ARTERIES
THYROID GLAND (BEHIND EYE)
OPHTHALMIC ARTERY
SHUNT BETWEEN CHOROID GLANDS
PSEUDOBRANCHIAL ARTERY
VENTRAL AORTA
AFFERENT BRANCHIAL ARTERIES
THYROIDEAN ARTERIES
BULBUS ARTERIOSUS
CORONARY ARTERY
HEART (VENTRICLE) ATRIUM (DORSAL)
HEPATIC VEINS
ABDOMINAL VEIN
HEPATIC PORTAL VEIN
GASTRIC ARTERY
PARIETAL VEIN
PARIETAL ARTERY

Figure 6. Diagram of a teleost heart (modified from Randall, 1968).

Figure 7. Records of blood pressures in the ventricle and bulbous, and blood flow in the ventral aorta of lingcod, Ophiodon elongatus (Stevens et al, 1972, modified from Randall, 1970).


atrium
sinus
venosus
bulbous
ventricle

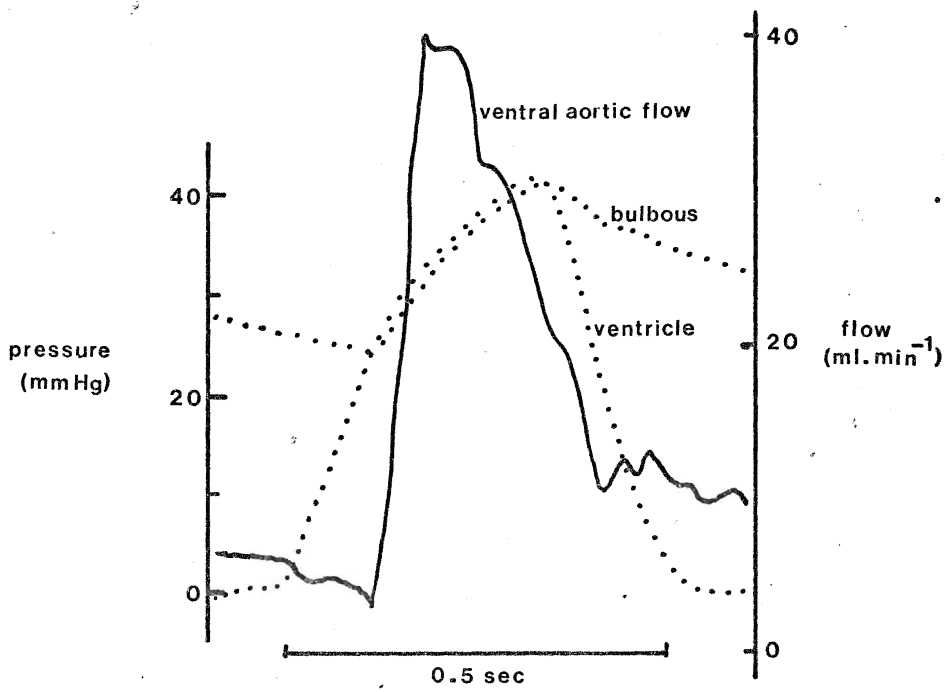

ventral aortic flow
bulbous
ventricle
pressure
(mm Hg)
40
20
0
40
20
0
flow
(ml. min^{-1})
0.5 sec

Figure 7 for lingcod, Ophidon elongatus. Stevens et al. (1972) have shown that elastic contraction of the bulbous during ventricular diastole plays a significant role in maintaining blood flow in the ventral aorta. This reduces the ventricular pressure required to maintain a given blood flow, and reduces the amplitude of the cardiac pulse due to ventricular contraction.

Among fish having a semirigid, non-compliant pericardium, ventricular contraction generates subambient intrapericardial pressures. This is transmitted to the atrium and sinus venosus, enhancing venous return. The occurrence of substantial cardiac "suction" forces has been well established in elasmobranchs; animals which possess the necessary rigid pericardium (Jones & Randall, 1978). Among teleosts, however, the heart is contained in a less rigid, more compliant pericardium, and this is structurally incompatible with the generation of negative intrapericardial pressures. Nevertheless, negative pressures have been recorded in the central veins and sinus venosus of teleosts (Jones & Randall, 1978). For a more thorough discussion of venous return and a complete list of references Randall (1970a), Satchell (1970), Johansen (1971), and Jones and Randall (1978) should be consulted.

(ii) Regulation of the cardiovascular system.

Regulation of cardiac activity in fish involves the integration of neural, aneural, and hormonal factors acting on the heart, and the branchial and systemic vasculature. Literature pertaining to cardiac regulation is fragmentary, and in some cases discrepant, making generalization difficult (Randall, 1968, 1970a; Johansen, 1971; Satchell, 1971; Jones & Randall, 1978). Some of these problems may arise from interspecific variation in the autonomic nervous systems of the species examined, particularly with regard to sympathetic innervation of the heart (Priede, 1974).

Recent histochemical and ultrastructural evidence (Yamauchi & Burnstock, 1968; Gannon & Burnstock, 1969) indicates that contrary to early beliefs, the teleost heart is actually doubly innervated by both parasympathetic (cholinergic) and sympathetic (adrenergic) efferent fibres. Both types are mediated by the vagus, but adrenergic components may also innervate the heart via the coronary arteries. Vagal innervation (adrenergic and cholinergic) is most densely distributed in the sinoatrial region, the primary centre of automatism, and to a lesser extent in the atrial area as well. In trout (Gannon & Burnstock, 1969) electrical stimulation of the cut vagus resulted in the typical negative ionotropic (myocardial contraction) and chronotropic (heart rate) responses. Stimulation following treatment with atropine, a cholinergic blocking agent, produced cardioacceleration which could be blocked by β-adrenergic blocking agents such as phenoxybenzamine. The balance of evidence, therefore, suggests that there is direct sympathetic innervation of the fish heart.

Vagal innervation of branchial and systemic vascular beds has been shown indirectly by histological and pharmacological studies (Campbell, 1970; Satchell, 1961). It has been proposed that vagal adrenergic innervation promotes vasodilation (vasopressor) in the gills and peripheral circulation, while the vagal cholinergic innervation leads to vasoconstriction or pressor response. Little is known, however, regarding the extent to which the vagus controls vascular resistance under resting, and more active conditions (Jones & Randall, 1978). Although direct evidence for neural control of vasomotor tone is lacking, Wood (1974b) has described regular oscillations in ventral and dorsal aortic blood pressures in unanesthetized rainbow trout similar to the Mayer vasomotor waves observed in mammals as a result of hemorrhagic hypotension (Guyton, 1966). In mammals this type of pressor response is attributed to oscillation within the negative feedback system

controlling pressure and flow under circumstances in which vascular pressure is reduced. In teleosts this phenomena was felt to be mediated via α-adrenergic receptors which could be directly activated by sympathetic innervation (Wood, 1974b). The elimination of the waves following α-adrenergic blockade supports this view (Wood, 1974b).

The nature of the afferent limb of the autonomic nervous system remains uncertain at present for, despite attempts to ascertain the type and location of afferent receptors, no clear picture has evolved (Randall, 1970a; Johansen, 1971; Satchell, 1961; Daxboek & Holeton, 1978; Smith & Jones, 1978). Efferent nervous activity has been found in the excised pseudobranch which possess both baroreceptor and chemoreceptor capabilities (Laurent, 1967, 1969; Laurent & Rouzeau, 1969, 1972). The pseudobranch, however, does not appear to be involved in the cardiovascular-ventilatory responses of intact fish to hypoxia (Hughes & Shelton, 1962; Saunders & Sutterlin, 1961; Randall & Jones, 1973; Bamford, 1974). Cardiac responses (bradycardia) are mediated by a peripheral oxygen sensitive chemoreceptor located in the anterodorsal region of the first gill arch (Daxboek & Holeton, 1978).

Available evidence suggests that receptors are present externally on the gills, and internally within the vascular system (both efferent and afferent to the gills). These monitor pertinent characteristics of water and blood respectively, and their stimulation generates action potentials initiating efferent vagal activity via the medulla. Although the exact nature of the stimulus activating any particular response is unclear, Satchell (1961) suggests that these receptors possess baroreceptor and/or chemoreceptor properties which respond to changes in a variety of blood and water parameters (including Po_2, Pco_2, pH, flow velocity) important in gas exchange.

Cardiac output can also be modified by intrinsic and extrinsic aneural factors. Bennion and Randall (1970, cited by Satchell, 1971) have reported

positive ionotropic and chronotropic responses in isolated rainbow trout hearts to increased perfusion pressure. Such responses suggest that, in vitro, at least, the trout heart obeys Starling's law. This states that the energy of myocardial contraction is a function of the length of the muscle fibres (i.e., the degree of stretch) at the onset of shortening. In fish, stroke volume will be a function of end diastolic volume which is determined by venous input pressure. Under strictly aneural conditions, therefore, stroke volume increases with venous return. The positive chronotropic response, however, is felt to be related to the pacemaker cells of the heart, since these depolarize more rapidly when stretched (Jensen, 1961). These intrinsic properties of the fish heart will obviously provide a basis for, at least, coarse modulation of cardiac activity (Satchell, 1961).

In the intact fish venous return can be regulated in a number of ways: (1) by changing total peripheral resistance; (2) by adjustment of intrapericardial pressure through altered cardiac activity; (3) by coordinated contraction of the skeletal muscles in the presence of a network of systemic valves; (4) in some teleosts, as a consequence of the presence of an elastic dorsal aortic ligament which acts as a pump in conjunction with body movement associated with swimming (Priede, 1975); (5) through mobilization of blood from storage organs (e.g., spleen) or venous reservoirs (Randall, 1970a).

Extrinsic aneural cardioregulation involves catecholamines secreted from chromaffin cells found in the veins leading to the heart (e.g., trout) and in the mycardium itself (e.g., skate and lungfish) (Gannon & Burnstock, 1969). These hormones act on adrenergic receptors throughout the cardiovascular system, including β_I adrenergic receptors in the gills (Wood, 1974a). The resulting vasodilation leads to a decrease in resistance, and alterations in the perfusion pathway through the gills. Intravenous injections of catecholamines do, in fact, cause vasodilation at the gills, while similar

injections of acetylcholine cause vasoconstriction of the branchial vasculature (Campbell, 1970). Recent studies (Davis, 1972; Hughes, 1972a; Morgan & Tovell, 1973; Wood, 1974a; Booth, 1978) have indicated that catecholamines can also decrease gill resistance through lamellar recruitment. Circulating catecholamines also act on α-adrenergic receptors in the systemic vascular bed, causing vasoconstriction and concomitant increase in peripheral resistance. In addition, through their action on β-adrenergic receptors in the heart a positive ionotropic and chronotropic response can be induced by alteration of myocardial Starling relationships (Bennion, 1968; cited by Randall, 1970a).

In the intact organism cardiac output is determined by the interaction of the neural and aneural parameters referred to above. Studies on intact organisms utilizing intravenous injections of vasoactive agents (e.g., epinephrine) are at best difficult to interpret. It has been proposed (Randall & Stevens, 1967; Satchell, 1971) that a baroreceptor reflex similar to that of the carotid sinus in mammals is present in some species of fish. Intravenous injection of adrenalin produced bradycardia, an increase in blood pressure, and a fall in cardiac output in lingcod (Stevens et al., 1962) and coho salmon (Randall & Stevens, 1967). Similar use of adrenalin following intrapericardial injection of atropine abolished the bradycardia. The changes in the cardiovascular system associated with adrenalin injections did not occur following α-adrenergic receptor blockage. These results could be explained by action of adrenalin on α-adrenergic receptors, resulting in an increase in blood pressure. Elevation in blood pressure could then elicit a pressor response via baroreceptors prompting decreased vagal sympathetic and/or increased vagal parasympathetic tone to the heart.

In higher vertebrates changes in cardiac output can usually be accounted for in terms of changes in vagal tone (Randall, 1970a; Johansen, 1971;

Satchell, 1961). Fish, however, exhibit interspecific differences in resting levels of inhibitory vagal tone, and also with respect to changes in the vagal inhibition associated with varying environmental conditions (e.g., temperature, hypoxia) and exercise (Randall, 1968; Jones & Randall, 1978). For example, there is a relatively high level of inhibitory vagal tone in resting cyprinids (e.g., carp), but normally active fish such as the salmonids (e.g., trout) do not exhibit inhibitory vagal tone during either rest or exercise under normoxic conditions (Randall, 1968). These animals, therefore, normally rely on sympathetic vagal input, and aneural mechanisms to modify cardiac output. However, under hypoxic conditions, an increase in parasympathetic vagal tone is observed for teleosts and elasmobranchs. It is evident from work with bilaterally vagotamized fish (Priede, 1974) that rainbow trout possess considerable capacity for aneural cardiac regulation.

Randall (1968, 1970a) concluded that in fish, control of cardiac activity is associated with large changes in stroke volume and limited alterations in heart rate. Stroke volume changes can be mediated by increased levels of circulating catecholamines and increases in venous return to the heart. Alterations in heart rate can be mediated by adrenergic and cholinergic vagal efferents, changes in temperature, and changes in the level of circulating catecholamines (Randall, 1970a).

(e) Gas Exchange

(i) General considerations

In order to satisfy oxidative requirements fish transfer large volumes of oxygen and carbon dioxide between the surrounding water and the tissues. Gas exchange involves six steps: (1) convective transport of the external medium to the gill surface; (2) diffusion across the lamellae; (3) convective transport by the blood; (4) diffusion across the microcirculatory walls; (5) diffusion through the interstitial and other extravascular, extracellular

compartments to the cells; (6) diffusion across the cell membrane and cytosol. These convective and diffusive processes must proceed at sufficient rates to meet the oxygen demands of the tissues, and to prevent carbon dioxide accumulation.

The rate at which gas molecules diffuse through the branchial or systemic exchangers will depend on; (1) the diffusion distance through the exchanger, (2) the surface area available for exchange, (3) the diffusion coefficient, and (4) the partial pressure gradient for the gas across the exchanger. It was noted earlier in discussing gill structure that fish are characterized by relatively short diffusion distances (1 to 6 μm), and exchange areas (~4.9 $cm^2 g^{-1}$) 10 to 60 times larger than the body surface area (Randall, 1970b).

Diffusion coefficients for oxygen and carbon dioxide in water and tissues are very low. The diffusion coefficient of CO_2, and therefore its rate of diffusion are, however, 20 to 30 times that for oxygen. Rate of diffusion through the respiratory exchanger is ordinarily assumed to be equal to that in water, the slowest medium encountered along the diffusion pathlength.

Direction and rate of diffusion are also governed by the partial pressure (or tension) gradient for each species of gas across the respiratory membranes. Fish normally maintain relatively large oxygen tension gradients across the gills; tensions equivalent to 40 to 100 mm Hg (Randall, 1970b). The carbon dioxide gradient in the reverse direction is much less (3 to 6 mm Hg), but this is compensated for, to some extent, by its larger diffusion coefficient. The literature contains few references to gas tensions in the tissues. In a study utilizing artificial gas pockets (Garey and Rahn, 1970), however, tissue gradients for oxygen were ~45 mm Hg in rainbow trout and ~20 mm Hg for carp. Those of carbon dioxide were 4.4 and 2.6 mm Hg in the trout and carp respectively.

Because it depends on random molecular events diffusion cannot, of

course, provide transport between respiratory surfaces and sites of use. Gases must be transported between the exchange sites, and over the respiratory membranes by convection or bulk flow processes by blood (perfusion) and water (ventilation). The rate of convective transport is related to; (1) the gas-carrying capacity of the medium, and (2) its viscosity or resistance to flow.

The high densities and viscosities of blood and water impose large demands on the ventilatory and circulatory systems. For teleosts, under resting conditions, the oxygen cost of operating the ventilatory muscles appears to be in the range of 5 to 15% of the total oxygen consumed (Cameron & Cech, 1970; Shelton, 1970; Jones & Schwazfeld, 1974). The efficiency of ventilation is equal to the oxygen equivalent of the work done at a particular ventilation volume divided by the total oxygen demand of the branchial muscles (Jones, 1971). The resting efficiency of the branchial muscles is low, with reported values being less than 2% (Davis & Randall, 1973; Jones & Schwazfeld, 1974). Mechanical efficiency will increase with power output up to a maximum value of 10%, after which efficiency again declines (Hughes & Saunders, 1970; Jones, 1970).

There appears to be no information in the literature regarding the metabolic costs of circulation or the efficiency of cardiac work in fish. Jones (1971) assumed a value of 20% for the resting efficiency of the teleost heart based on mammalian studies. If this value is applied to the data of Kiceniuk and Jones (1977), the oxygen cost of operating the cardiac pump is 2 to 4% of the total oxygen consumed in resting rainbow trout. Similar calculations yield a value of 5.2% for carp (Garey, 1970). The oxygen cost of operating the cardiac pump at rest in man is 8% (Rothe, 1966; cited by Garey, 1970) a value in good agreement with those devised for fish on the basis of work output by heart.

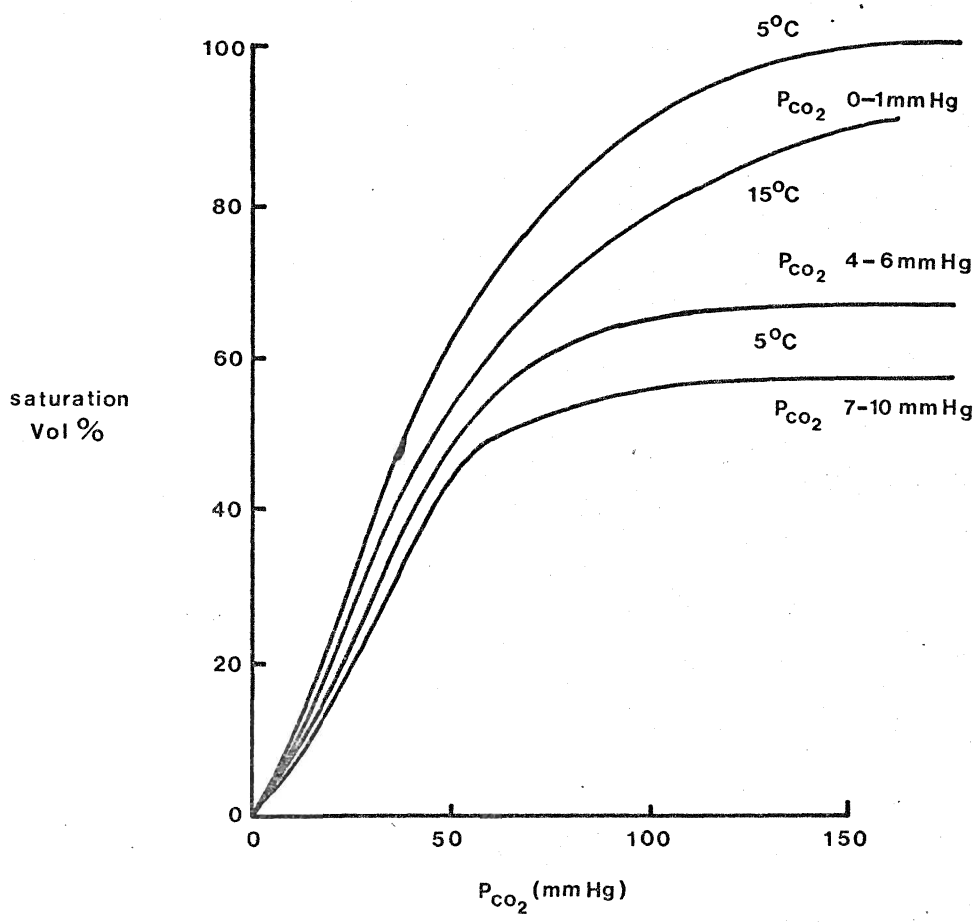
(ii) Oxygen transport

The oxygen-carrying capacities of the two respiratory media are quite different. The oxygen content of water is significantly affected by temperature and ionic strength (Randall, 1970b). The saturation oxygen content of fresh water is between 0.5 and 1.0 Vol %. The oxygen content of fully saturated blood is 4 to 10 Vol %. In fact, blood efferent to the gills is usually 95% saturated with oxygen in rainbow trout (Holeton & Randall, 1967b; Stevens & Randall, 1967b). Fish must therefore ventilate large volumes of water over the gills, relative to the blood flow through the gills, in order to fully saturate the blood at all times. The ratio of water to blood flow in fish is always high, with a value of 80:1 reported for trout (Stevens & Randall, 1967b).

The disparity in the oxygen carrying capacities of blood and water is due to the transport pigment hemoglobin found in the red cells. All of the oxygen present in fresh water is in physical solution and obeys Henry's law.* The oxygen content of blood, however, is not linearly related to the partial pressure of oxygen present. The relationship between oxygen content and partial pressure is described by the oxygen dissociation curve (Fig. 8). Physically dissolved oxygen accounts for less than 5% (<0.5 Vol %) of the total oxygen in the blood, the remaining 95% being bound to hemoglobin. The cooperative nature of oxygen/hemoglobin binding is reflected in the sigmoidal nature of the curve. The steeper the slope, the larger the amount of gas which can be transferred per unit difference in oxygen tension across the gills (Randall, 1970b). In summary then, the oxygen content of water is low (0.5 - 1.0 Vols %) but the oxygen tension gradient into the blood is high (40 to 100 mm Hg Po_2).

*the amount of gas that dissolves in a given quantity of a liquid at constant temperature is directly proportional to the partial pressure of the gas above the solution (Barrow, 1972).

Figure 8. Oxygen dissociation curves for the blood of rainbow trout (Salmo gairdneri) at different carbon dioxide tensions and different temperatures (Beaumont, 1968; modified from Randall, 1970b).


5°C
P_{CO_2} 0–1mm Hg
15°C
P_{CO_2} 4–6mm Hg
5°C
P_{CO_2} 7–10 mm Hg
saturation
Vol %
100
80
60
40
20
0
0
50
100
150
P_{CO_2} (mm Hg)

The properties of blood are such that large amounts of oxygen (4 to 10 Vol %) can be transferred across the respiratory membrane without large changes in the oxygen tension of the blood.

Fish bloods frequently exhibit high affinity for oxygen and are 95% saturated at partial pressures which are relatively low compared to the oxygen tension of inspired water (150 mm Hg Po_2). Randall (1970b) has compiled P_{50} values (partial pressure for 50% saturation) for various species of fish. The P_{50} value for trout at 5°C is 38 mm Hg Po_2 (Beaumont, 1968; cited by Randall, 1970b) and for carp at 10°C is 4 mm Hg (Garey, 1967 cited by Randall, 1970b). Assuming that the rate of hemoglobin oxygenation is not limiting, the partial pressure for oxygen in blood at the gills will not rise above 95 - 100 mm Hg until the hemoglobin is fully loaded in trout. The countercurrent exchange process proposed for teleost gills (Hughes & Shelton, 1962; Hughes 1964, 1972 a,b; Hills & Hughes, 1970; Piiper & Scheid, 1972, 1975) whereby interlamellar water and intralamellar blood flow in opposite directions, serves to maintain large tension gradients along the entire length of the lamellar exchange surface. Such maintenance of diffusion gradients maximizes oxygen transfer per unit exchange area.

Interspecific variations are found in oxygen transport. For example, the oxygen tensions of venous and arterial blood are less for carp than rainbow trout (Table 1). The mean oxygen tension gradients across the gills, however, are similar (Carp: ΔPo_2 ~49 mm Hg; trout: ΔPo_2 ~65 mm Hg). At normal carbon dioxide levels (1 to 2 mm Hg, Pco_2) arterial blood in carp is 95% saturated at oxygen tensions of 20 to 25 mm Hg (Garey, 1967). The carp is characterized by lower ventilation volumes, higher utilization*, and higher hemoglobin-oxygen affinity than is the trout. The high affinity

*% of oxygen removed from inspired water

Table 1. Tissue and blood gas tensions in trout (Salmo gairdneri) and carp (Cyprinus carpio). Blood values for trout were taken from Stevens and Randall (1967b) and those for carp from Garey (1967). (Table is taken from Garey and Rahn, 1970).

	O_2 tension		CO_2 tension	
	Trout	Carp	Trout	Carp
water	134	108	-.--	-.--
arterial	85	33	2.30	3.10
tissue	40	13	6.70	5.70
mixed venous	19	3	5.70	3.60

carp hemoglobin, therefore, maintains intralamellar blood oxygen tension at low levels (20 to 30 mm Hg, Po_2) until the hemoglobin is fully loaded. This serves to maintain the oxygen tension gradient into the blood in the face of concomitant decreases in the oxygen tension of interlamellar water. In the trout, however, the lower oxygen affinity of the hemoglobin allows blood oxygen tension to increase as blood passes through the lamellae, while greater ventilation volumes and lower utilization serve to maintain the Po_2 levels of water passing over the gills at high levels (130 to 140 mm Hg, Po_2). These factors, in combination with the counter current exchange process, insure that large oxygen-driving tensions are maintained at the gills under most circumstances, and illustrate the distinct species differences in how this is achieved.

Total oxygen transport capacity in most fish blood equals arterial carrying capacity and is between 4 and 12 Vols %. In the specific case of salmonids arterial oxygen-carrying capacities range from 9 to 12 Vols %. The total transport capacity depends on the carrying capacity of the blood and the conditions at the gills for gas exchange. An increase in the amount of hemoglobin present (due to an increase in hemoglobin per cell, or an increased number of red cells) increases potential transport capacity. Decreases in erythrocyte volume increase the rate at which oxygen is taken up by the blood and compensate for reductions in branchial transet time as cardiac output is increased. Increases in hemoglobin-oxygen affinity lower the partial pressure of oxygen necessary to saturate the blood, and under certain conditions facilitate both the rate and amount of oxygen uptake.

Effective oxygen transport - the amount of oxygen which is actually delivered to the tissues - is variable. Values for effective transport, as indicated by arterio-venous oxygen differences are given in Table 2 for resting rainbow trout. Variability in venous oxygen content can be attributed

Table 2. Total and effective oxygen transport in resting rainbow trout (Salmo gairdneri).

Temp. °C.	Oxygen content (Vols %) arterial	venous	arterio-venous diff.
4 - 8*	9 - 10	3 - 4	6 - 7
2 - 13**	9 - 12	1 - 3	6 - 11
15***	9 - 10	6 - 7	3 - 4

* Stevens and Randall (1967b)

** Itazawa (1970)

*** Holeton and Randall (1967b)

to differences in oxygen demand, cardiac output, and oxygen-carrying capacity of the blood in relation to the particular experimental situation employed. Temperature, oxygen levels in the water and activity are all important in this regard.

In rainbow trout exposed to hypoxic conditions (Po_2: 30 mm Hg) the oxygen content of dorsal aortic blood was only 3 to 4 Vol % (37% saturated), while that of ventral aortic blood was less than 1 Vol % (Holeton & Randall, 1967b). The fish, apparently, was unable to fully saturate its blood, and therefore decreased venous oxygen content in order to maintain the effective amount of oxygen delivered to the tissues. This type of response is well within the storage capacity of the blood which remains 70% saturated (6 to 7 Vol %) under normoxic conditions. Under different environmental circumstances (e.g., temperature) or when forced to increase activity, increased metabolic oxygen demand might well be satisfied in a similar fashion.

Ability to increase effective oxygen transport capacity requires that fish be able to modify exchange conditions at the tissue level. The factors governing such exchange are less well understood than are those at the branchial exchanger level. Garey and Rahn (1970), however, determined tissue gas tensions in rainbow trout and carp using the artificial gas pocket technique developed for mammals (Table 1). Tissue oxygen tensions in both species lie between values for arterial and mixed venous oxygen tensions. Oxygen tension gradients are reduced relative to those across the gills. Deoxygenation of hemoglobin can be facilitated at the tissues by modulation of hemoglobin-oxygen affinity. Teleost hemoglobins sometimes exhibit a marked Bohr (increase in the level of carbon dioxide, or a decrease in pH reduces hemoglobin-oxygen affinity) and Root effects (reduction of overall oxygen-carrying capacity prohibiting complete saturation) as shown in Fig. 8 (Randall, 1970b). The magnitude of these effects is normally most pronounced

at normal carbon dioxide tensions of 1 to 5 mm Hg (Holeton & Randall, 1967b). Transfer of oxygen in the tissues, therefore, is facilitated by reduced oxygen-hemoglobin affinity due to the operation of a significant Bohr and Root effect in the presence of increased carbon dioxide levels and decreased pH levels (Randall, 1970b; Johansen, 1971). The amount of oxygen released when these affinity influencing factors are operating is much greater than that which occurs due to the reduction in the oxygen tension in blood circulating through the systemic system alone. From an interspecific point of view it is interesting to note that tissue oxygen tensions in carp (13 mm Hg) are considerably lower than those of rainbow trout (40 mm Hg). Garey and Rahn (1970) have shown, however, that at these oxygen tensions venous blood draining the tissues is 60 to 70% saturated for both species. They suggest that the disparity in tissue oxygen tension is a result of the displacement of the oxygen dissociation curve to the right in trout as compared to carp in accordance with the lower affinity trout hemoglobin.

(iii) Carbon dioxide transport

The elimination of carbon dioxide produced in the tissues does not pose as serious a problem for fish, as does the transport of oxygen. Carbon dioxide transport in the blood is facilitated by (1) a diffusion coefficient which is 20 to 30 times that for oxygen, and (2) the chemical conversion of dissolved CO_2 into various transport forms. There is a paucity of data in the literature with regards to blood CO_2 levels. The total transport capacity- the total amount of carbon dioxide present in the blood - in trout is, however, approximately 15 - 25 Vol % (Holeton & Randall, 1967b; Stevens & Randall, 1967b; Randall, 1970). The effective transport capacity - the amount of carbon dioxide removed from the blood via the gills - under resting conditions is 5 to 10 Vols % (Stevens & Randall, 1967b).

Carbon dioxide is transported in four different forms: (1) as physically dissolved molecular carbon dioxide, (2) as carbonic acid, (3) as bicarbonate produced from the dissociation of carbonic acid, and (4) as carbamino compounds (primarily those involving hemoglobin). Although information is lacking for fish, the relative contribution of each form of carbon dioxide to the total and effective carbon dioxide transport capacity has been determined for mammals (Table 3). The major differences between mammalian and fish blood which could be expected to result in differences in carbon dioxide transport are the (1) lower temperatures, (2) reduced carbon dioxide tensions, (3) nucleation of erythrocytes, and (4) lower levels of hemoglobin found in fish (Albers and Pleschka, 1967). It has been concluded (Albers & Pleschka, 1967) that the higher transport capacities in mammals as compared to fish can be accounted for principally in terms of the lower concentration of hemoglobin in fishes, as this is the primary protein buffer in blood.

Assuming that the analogy with mammals is valid, plasma bicarbonate probably constitutes the major component of both total and effective carbon dioxide transport. Physically dissolved molecular carbon dioxide is maintained at low levels in the blood by the action of the enzyme carbonic anhydrase present in the red cells of fish (Dejours, 1967; Haswell, 1977; Smeda & Houston, 1979). The uncatalysed reaction between dissolved carbon dioxide and water in plasma to form carbonic acid is too slow (in the order of seconds) to be useful in terms of transport. The reaction as catalysed by carbonic anhydrase, however, proceeds at 200 to 300 times the rate of the noncatalysed reaction, producing large amounts of carbonic acid, which then dissociates to bicarbonate and hydrogen ions. The bicarbonate diffuses out of the red cell in an exchange for chloride while most of the hydrogen ion is buffered by hemoglobin. In the intact rainbow trout 10 to 20% of total bicarbonate is effectively eliminated as blood passes through the gills (Haswell, unpublished observations; cited

Table 3. Relative contribution of various carbon dioxide components to total and effective CO_2 transport by the blood in man. (values taken from Guyton, 1966).

Transport form	Total transport (%)	Effective transport (%)
dissolved	5	7
bicarbonate	90	63
carbamino compounds	5	30

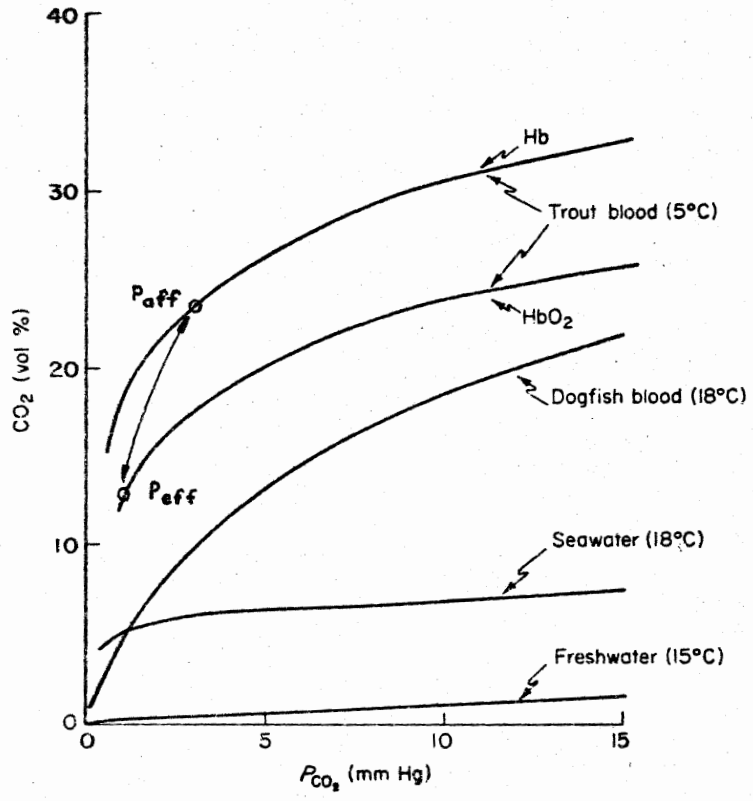
Haswell & Randall, 1978).

In addition to its reacting with water, carbon dioxide can combine with hemoglobin to form carbamino-hemoglobin. This component of carbon dioxide accounts for 30% of the effective carbon dioxide transport in mammals (Guyton, 1966), but there is no direct evidence for the presence of carbamino-hemoglobin in fish (Albers, 1970).

Total blood carbon dioxide depends principally on the partial pressure of carbon dioxide dissolved in the blood. This tension is maintained at low levels in teleost blood. Reported values for carbon dioxide tensions in venous blood range from 2.5 to 6.0 mm Hg. and in arterial blood from 1.0 to 2.3 mm Hg (Holeton & Randall, 1967b; Stevens & Randall, 1967b; Albers, 1970; Cameron, 1971). The physiological significance of these reduced carbon dioxide tensions can be illustrated by reference to oxygen dissociation curves for teleosts (e.g., Fig. 8). The hemoglobin of rainbow trout exhibits strong Bohr and Root effects. At 5°C, Po_2 levels of 100 mm Hg, and Pco_2 levels similar to those found in venous blood (4 to 6 mm Hg), trout blood is only 60% saturated with oxygen, i.e., the affinity of hemoglobin for oxygen is decreased (Beaumont, 1968; cited by Randall, 1970b). However, at 5°C, Po_2 levels of 100 mm Hg, and Pco_2 similar to arterial blood (i.e., efferent to the gills, 0 - 1 mm Hg) the blood achieves 95% saturation (Beaumont, 1968; cited by Randall, 1970b). The overall efficiency of the carbon dioxide transport system is indicated by the fact that blood efferent to the gills is seldom less than 95% saturated (Randall, 1970).

The relationship between carbon dioxide content (all forms and carbon dioxide tension is expressed by the equilibrium curves (Fig. 9) for oxygenated and deoxygenated blood in the trout and dogfish. The slope of this curve is steep in regions of low, and for fish, physiological carbon dioxide

Figure 9. Carbon dioxide dissociation curves for the blood of rainbow trout and dogfish. (modified from Randall, 1970b).


40
30
20
10
0
CO_2 (vol %)
Hb
Trout blood (5°C)
HbO_2
Dogfish blood (18°C)
Seawater (18°C)
Freshwater (15°C)
P_{aff}
P_{eff}
0
5
10
15
P_{CO_2} (mm Hg)

tensions, i.e., from 1 to 5 mm Hg. By this large amounts of carbon dioxide can diffuse down the tension gradient without causing large changes in carbon dioxide partial pressure on either side of the exchanger. This greatly facilitates carbon dioxide transfer in the tissues and across the gills.

In addition, deoxygenated trout blood has a greater carbon dioxide capacity than oxygenated blood. This is indicated by the displacement of the dissociation curves (Fig. 9). Dogfish blood does not exhibit this effect. In the case of salmonids this phenomena approximately doubles the amount of carbon dioxide which can be effectively transported by the venous blood, and eliminated by the branchial exchanger. This difference between oxygenated and deoxygenated hemoglobin is termed the Haldane effect, and involves two factors. (1) Deoxyhemoglobin is a weaker acid than oxyhemoglobin (i.e., the pKa is greater in the deoxygenated state) and buffers at a higher pH than oxyhemoglobin. The pH of deoxygenated blood in the tissues is thus greater than oxygenated blood leaving the gills for any given carbon dioxide tension. Deoxyhemoglobin, therefore, enhances bicarbonate formation in the tissues since it has a greater affinity for H^+ ions and thereby increases the total carbon dioxide capacity of deoxygenated blood as compared to oxygenated blood at any given carbon dioxide tension. (2) Deoxyhemoglobin, being a weaker acid, binds more carbamino compounds than oxyhemoglobin (Riggs, 1970). Involvement of carbamino compounds and the Haldane effect, however, is not seen in all fish, although they do appear to play a role in CO_2 transport by salmonids (Randall, 1970b; Riggs, 1970).

Diffusion of carbon dioxide across respiratory membranes, therefore, takes place over small tension gradients, and is facilitated by the relatively high diffusion coefficient and the steepness of the dissociation curves. In the capillaries, physically dissolved molecular carbon dioxide diffuses into the blood, and then into the red cell where it is converted into the various

chemical forms. The red cell thus acts as a carbon dioxide sink for diffusion from the tissues.

In the gills dissolved carbon dioxide readily diffuses down small tension gradients into the water. The situation here is more complex because of the various forms of carbon dioxide present, and the literature does not indicate that there is general agreement as to the importance of dissolved carbon dioxide (Randall, 1970b; Haswell & Randall, 1978) or bicarbonate (Dejours, 1969) in the elimination of carbon dioxide at the gills. The possible importance of bicarbonate has been indicated by the presence of a branchial ion exchange process of H^+ for Na^+, and HCO_3^- for Cl^- in rainbow trout (Kerstetter, et al. 1970; Kerstetter & Kirshner, 1972) and other teleosts (Dejours, 1969; Maetz, 1971). This exchange process appears to be obligatory in that carbon dioxide evolution depends to some extent on its operation (Dejours, 1969).

Carbon dioxide loss at the gills is also enhanced by the curvilinear nature of the dissociation curves of blood and water at low carbon dioxide tensions. Large amounts of carbon dioxide (all forms) can be transported across the exchanger without reducing the tension gradient significantly. In addition, if the ratio of carbon dioxide evolved-to-oxygen taken up by the gills is assumed to be unity, the disparities in the solubilities of the two gases necessitate a high ventilation/perfusion ratio (Rahn, 1966). Under these conditions water acts as an effective carbon dioxide sink, and the tension of carbon dioxide in water remains close to ambient. The exchange ratio (R) would be expected to vary in response to shifts in intermediary metabolism associated with the utilization of different substrates. Although little data exists R is generally assumed to be close to unity (Randall, 1970b; Hughes and Morgan, 1973), and Morris (1967) has reported R values for the yellow bullhead (Ictaluras natalis), and a cichlid (Aequidens portagrenosis) in this range which are close to one.

In summary, gas exchange in fish is facilitated by the mutual interaction of the transport systems for oxygen and carbon dioxide. The carrying capacities of blood for oxygen and carbon dioxide are closely related, and dependent upon conditions at the tissues and gills for exchange. It is difficult, in terms of overall gas exchange theory, to separate Bohr, Root and Haldane effects, since all of these processes contribute to the transport state of the blood at any given time.

(f) Analysis of Branchial Gas Exchange.

In recent years respiratory gas exchange, particularly that of teleost fish, has been the subject of a number of reviews (Fry, 1957; Randall, 1970b; Johansen, 1971; Hughes & Morgan, 1973) and theoretical analyses (Hughes & Shelton, 1962; Hughes, 1964, 1966; Rahn, 1966; Randall, Holeton, & Stevens, 1967; Taylor Houston, & Hogan, 1968; Jones, Randall & Jarman, 1970). Equations derived by these authors have made quantitative analysis of the gas exchange process possible.

A major difficulty in studying gas exchange across the gills lies in obtaining reliable estimates of the physico-chemical properties of water and blood. Sampling of blood and water afferent and efferent to the gills can be done, however, through the use of catheters emplaced in the buccal and opercular chambers (Saunders, 1961), and dorsal and ventral aortae (Holeton & Randall, 1967b). A summary of reported values for the respiratory characteristics of blood and water is given in Table 4. All of these apply to the rainbow trout as this species has received particular attention in such studies.

From the previous discussion of oxygen and carbon dioxide transport, it will be apparent that gas exchange is influenced by the flow rates of the respiratory media on both sides of the exchanger. Gas tensions have been used to calculate ventilatory/circulatory flow rates by means of the Fick

Table 4. Summary of reported values for respiratory characteristics of blood and water of rainbow trout (*Salmo gairdneri*).

Temp.°C	Inspired water O_2 tension mmHg.	Expired water O_2 tension mmHg.	Arterial O_2 tension mmHg.	Venous O_2 tension mmHg.	Arterial O_2 content Vols.%	Venous O_2 content Vols.%	Arterial-venous O_2 content Vols.%	% Saturation arterial %	% Saturation venous %	Hematocrit arterial %	Arterial CO_2 tension mmHg.	Venous CO_2 tension mmHg.	pH arterial	pH venous	References
5	150	75	89	23	8.0	---	---	95-100	---	23	---	---	---	---	Holeton, (1971)
4-8	134	121	85	19	9.0-10.0	3.0-4.0	5.0-7.0	100	38	--	2.3	5.7	---	---	Stevens and Randall, (1967b)
9	160	86	130	32	---	---	---	85-100	---	23	3.0	3.0	---	---	Davis and Cameron, (1970)
9-10.5	153	103	137	33	10.4	7.1	3.3	97	70	23	---	---	7.93	7.96	Kiceniuk and Jones, (1977)
2-13	100-199	---	---	---	10.7	1.3	9.4	---	---	--	---	---	---	---	Itazawa, (1970)
10	---	---	---	---	---	---	---	---	---	--	1.8	3.8	7.90	---	Cameron and Randall, (1972)
10-13.5	153	118	117	32	---	---	---	---	---	--	3.0	3.0	---	---	Davis and Cameron, (1970)
15	155	97	122	35	8.5	6.3	2.2	95-100	70	20-30	1.0-1.5	2.5	7.70	7.7	Holeton and Randall, (1967b)
16	151	---	---	---	6.0-9.0	3.0-4.0	2.0-6.0	92	---	--	---	---	---	---	Heath and Hughes, (1973)

principle. A summary of calculated cardiovascular and ventilatory parameters is given in Table 5 for rainbow trout. Recent authors (Davis & Watters, 1970) have criticized this indirect means of analysis on the grounds that water sampled from opercular cannulae does not provide reliable estimates of mixed expiratory flow from the gills. For this reason direct methods of measuring ventilation volume have been developed (Hughes & Shelton, 1958, 1962; Davis & Cameron, 1970; Davis, 1961) whereby light membranes are used to separate inspired from expired water.

The ventilation/perfusion ratio ($\dot{V}_G/\dot{Q}$) is commonly used by mammalian physiologists to analyse gas exchange. The $\dot{V}_G/\dot{Q}$ ratio, which is close to one in man, is considerably greater for fish. The $\dot{V}_G/\dot{Q}$ ratio for rainbow trout, although variable, appears to be between 2:1 and 10:1 (Table 5). A high $\dot{V}_G/\dot{Q}$ ratio would be expected in fish on theoretical grounds due to the low solubility of oxygen in water (Rahn, 1966). The effect of changes in this ratio on gas exchange under varying conditions, however, has never been extensively analysed (Randall, 1970b). Clearly, the $\dot{V}_G/\dot{Q}$ ratio will depend on the conditions for exchange at the secondary lamellae where variations in flow can be achieved by changes in stroke volume and frequency. Although there is little data pertaining to water and blood flow at the gills, attempts have been made to illucidate the relationship between heart rate and ventilatory movements in fish. In teleosts there does not seem to be a particular phase in the ventilatory cycle when the heart beats under normal conditions (Hughes, 1961, 1964). An increase in cardio-ventilatory synchrony has been observed under anesthesia or hypoxic conditions (Randall & Smith, 1967), as well as with temperature change (Heath & Hughes, 1961). Variations in ventilation and perfusion are thought to be related to attempts to optimize conditions for gas exchange at the gills. A better understanding of the factors involved in the control of the distribution and flow rate of blood and water at the

Table 5. Summary of reported ventilatory and cardiovascular data for rainbow trout (Salmo gairdneri).

Temp. °C	Weight kg.	O_2 consumption* ($\dot{V}_{O_2}$) mg/kg/hr	% Utilization (%U) %	Ventilatory flow ($\dot{V}_G$) ml/kg/min	Cardiac output ($\dot{Q}$) ml/kg/min	$\dot{V}_G/\dot{Q}$	Ventilation rate (VR) No./min	Cardiac rate (CR) No./min	Vent. stroke vol. (Vsv) ml	Cardiac stroke vol. (Csv) ml	Capacity rate ratio	Transfer factor ml O_2/kg/min/mmHg	References
5	0.3-0.6	39	50	125[F]	---	---	47	29	---	---	---	0.0086	Holeton, (1971)
4-8	0.2-0.4	36	10	1428-2855[F]	15-30.0	95	78	47	7.30	0.15	5.0	0.0059	Stevens and Randall, (1967b)
9	0.2	55	45	171[om]	18.3	10	74	63	0.50	0.30	1.2	0.0160	Davis and Cameron, (1970)
9-10.5	0.9-1.5	45	33	211[F]	17.6	12	--	37	---	0.46	---	0.0130	Kiceniuk and Jones, (1977)
8-12	0.2	45-55	46	185[om]	31.0	5.8	74	--	0.50	---	---	------	Davis, (1971)
12	---	53	58	---	19.3	---	--	63	---	---	---	0.0170	Davis and Cameron, unpublished, cited by Cameron and Davis, (1970)
13.5	0.4-0.6	40-60	60	100-200[F]	---	---	40-60	--	1.0-3.0	---	---	------	Hughes and Saunders, (1970)
10-13.5	0.26	56	44	173**	77.0	2.2	--	49	---	0.31	---	0.0078	Davis and Cameron, (1970)
14	0.2	63	45	200[om]	---	---	77	--	0.59	---	---	------	Davis and Randall, (1973)
14	0.2	93	55	300[om]	---	---	78	--	0.81	---	---	------	Davis and Randall, (1973)
15	0.4-0.6	110	---	---	---	---	63-102	52	---	---	---	------	Heath and Hughes, (1973)
12-18	0.2-0.3	100-120	55	274[F]	65-100	2.7-4.2	80	78	0.7-1.0	1.25	2.5-4.0	0.0310	Holeton and Randall, (1967b)

* calculated for fish under resting conditions, termed "routine oxygen uptake" (Fry, 1957).

** this value refers to the flow rate at which the gills were perfused.

F - $\dot{V}_G$ calculated by the Fick principle from oxygen uptake data.

om - $\dot{V}_G$ measured directly using oral membrane.

gills is, however, required before the ventilation-perfusion relationship can be appreciated.

The most frequently used measure of exchanger efficiency in fish is "utilization", the fraction of oxygen removed from water during passage over the gills.

$$\%U = \frac{Pinsp - Pexp}{Pinsp} \times 100$$

Reported values for utilization are generally variable. For rainbow trout (Table 5) utilization estimates range from 10 to 60% under different conditions although most are close to 50%. Van Dam (1938, cited by Hughes & Morgan, 1973) obtained values of 70 and 80% for trout and eel. These high levels of oxygen extraction are usually attributed to the operation of the teleost gills as a counter current exchanger. In such systems it is theoretically possible, given infinite exchange area, that the oxygen tension of blood leaving the gills will equal that of water entering the gill sieve. It appears that this does not occur (Table 5), but the fact that the oxygen tensions of dorsal aortic blood are usually higher than those of water leaving the gills lends considerable support to the theory that such a system is employed.

Saunders (1962) and Randall (1970b) have proposed that utilization will vary with changes in the amount of water actually involved in gas exchange. Several studies (Saunders, 1962; Hughes, 1966; Holeton & Randall, 1967b) have found that utilization decreases as ventilation volume ($\dot{V}_G$) increases. In some cases, however, utilization has been found to remain constant with increasing $\dot{V}_G$ (Stevens & Randall, 1967b; Davis & Cameron, 1970; Kiceniuk & Jones, 1977). Randall (1970b) has proposed that changes in utilization are dependent upon the relative volumes of the "dead spaces" in the water passing over the gills. Randall divided total flow into the following components:

(1) a diffusional dead space, in which water remains in contact with the gill epithelium for too short a time for blood and water gas tensions to equilibrate; (2) a distributional dead space, associated with ventilation-perfusion relationships such that more water is delivered to particular gill areas than is necessary to saturate the blood with oxygen; (3) an anatomical dead space, consisting of water taking a non-respiratory pathway through the gill sieve, i.e., axial flow between the tips of the filaments.

Water flowing over the gills, therefore, can be divided into three components: (1) the respiratory volume which is actually involved in gas exchange; (2) the residual volume, consisting of very low velocity water, in contact with the walls of the buccal and opercular chambers; (3) the shunt volume passing over the gills, but not actually involved in gas exchange.

Davis and Randall (unpublished data) have analysed dead space phenomena in the gills of rainbow trout using the data of Davis and Cameron (1970). For resting, non-swimming animals with oral membranes attached, total shunt volume accounted for 30% of $\dot{V}_G$ over the range, 44 to 120 mls. min^{-1}. Diffusion and anatomical dead spaces accounted for only a small portion (<5%) of total flow. The distributional dead space made up the major portion of total shunt.

Several factors, therefore, are involved in determining utilization at the gills. Increases in $\dot{V}_G$ will, for example, produce increases in diffusional dead space (15% of total flow at 300 mls. min^{-1}) and this impedes oxygen uptake. Anatomical dead space is small at rest, and remains constant at low to moderate $\dot{V}_g$ (<300 mls. min^{-1}). Thus utilization (and gill resistance) does not change (Davis & Randall, 1973a). At high $\dot{V}_G$, however, anatomical dead space increases, and utilization then decreases. Distributional dead space decreases with increasing $\dot{V}_G$ as the number of secondary lamellae

perfused increased (Davis, 1972; Booth, 1978). At high $\dot{V}_G$ values distributional dead space becomes zero with the gills completely perfused, but the anatomical and diffusional dead space volumes are very large and account for 40 and 30% respectively of total flow. In spite of the limitations observed for high $\dot{V}_G$ Davis and Randall (1973a) concluded that the branchial exchanger of rainbow trout has evolved in such a way that dead space phenomena does not limit gas exchange at the lower $\dot{V}_G$ levels normally employed (<300 mls. min^{-1}).

Any attempt to assess branchial exchanger performance in terms of utilization, however, is limited due to the complex set of interrelated variables which make up this term (Hughes and Shelton, 1962). It was this problem which prompted Hughes and Shelton (1962) and Hughes (1966) to analyse the branchial exchanger in terms of the effectiveness of transfer; an approach derived from studies on countercurrent heat exchangers. Effectiveness is defined as the ratio of the gas actually transferred to the maximum rate of gastransfer possible. The latter can be realized only in a countercurrent exchanger of infinite area. The derivation of the concept of effectiveness involves mass transfer equations between water and blood as follows (from Hughes & Morgan, 1973; see Table 6 for explanation of symbols).

for oxygen

$$\dot{V}_{o_2} = V_G \, \alpha w, o_2 \, (Pinsp, o_2 - Pexp, o_2)$$
$$\dot{V}_{o_2} = Q \, \alpha b, o_2 \, (Peff, o_2 - Paff, o_2)$$

for carbon dioxide

$$\dot{V}_{co_2} = \dot{V}_G \, \alpha w, co_2 \, (Pexp, co_2 - Pinsp, co_2)$$
$$\dot{V}_{co_2} = Q \, \alpha b, co_2 \, (Paff, co_2 - Peff, co_2).$$

Table 6. List of symbols employed in the analysis of gas exchange.* (taken from Hughes and Morgan, 1973).

A	area of respiratory surface (mm^2)
$\alpha_{w,\,o_2}$	solubility (absorption) coefficient for oxygen in water (ml O_2 L^{-1} mm Hg $P_{o_2}^{-1}$)
$\alpha_{b,\,o_2}$	equivalent overall solubility coefficient for oxygen in blood (ml O_2 l^{-1} mm Hg $P_{o_2}^{-1}$)
C_b	oxygen transport capacity rate of blood (ml O_2 min^{-1})
C_w	oxygen transport capacity rate of water (ml O_2 min^{-1})
T_{o_2}	diffusing capacity of the gills (ml O_2 kg^{-1} min^{-1} mm Hg^{-1})
E_b	effectiveness (%) of transfer of a gas to or from blood
E_w	effectiveness (%) of transfer of a gas to or from water
K	Krogh permeation coefficient (ml min^{-1} cm^{2-1} um^{-1} mm Hg^{-1})
P_{aff}	partial pressure of gas (O_2) in blood going to the gills in the afferent branchial arteries
P_{eff}	partial pressure of gas (O_2) in blood leaving the gills in the efferent branchial arteries
ΔP_G	difference between the mean tensions of gas (O_2) in the water and blood entering and leaving the gills $= \frac{1}{2}\,[(P_{insp} + P_{exp}) - (P_{eff} + P_{aff})]$ $= \frac{1}{2}\,[(P_{insp} - P_{eff}) - (P_{exp} + P_{aff})]$
ΔP_{o_2}	difference in O_2 tension, e.g. $P_{insp} - P_{eff}$
$\dot{Q}$	cardiac output (ml min^{-1})
t	thickness of the barrier separating the water from the blood
%U	utilization - relative volume of oxygen removed from water during its passage over the gills, usually expressed as a percentage of O_2 content of inspired water.
$\dot{V}_G$	ventilation volume - volume of water pumped over the gills in unit time (ml kg^{-1} min^{-1})
$\dot{V}_o$	oxygen consumption - volume of oxygen transferred across the gills in unit time (ml O_2 kg^{-1} hr^{-1})

* Table is constructed with respect to oxygen. Symbols for parameters relating to carbon dioxide would have subscript CO_2.

The effectiveness of transfer can then be described by the following equations:

(i) the effectiveness of oxygen removal from the water

$$Ew, o_2 = \frac{(Pinsp - Pexp)\ \alpha w, o_2}{(Pinsp - Paff)\ \alpha w, o_2} \times 100$$

(ii) the effectiveness of oxygen uptake by the blood

$$Eb, o_2 = \frac{(Peff - Paff)\ \alpha b, o_2}{(Pinsp - Paff)\ \alpha b, o_2} \times 100$$

(iii) the effectiveness of carbon dioxide removal from the blood

$$Eb, Co_2 = \frac{(Paff - Peff)\ \alpha b, Co_2}{(Paff - Pinsp)\ \alpha b, Co_2} \times 100$$

(iv) the effectiveness of carbon dioxide uptake by the water

$$Ew, Co_2 = \frac{(Pexp - Pinsp)\ \alpha w, Co_2}{(Paff - Pinsp)\ \alpha w, Co_2} \times 100$$

The effectiveness of transfer depends on the following factors:

(1) <u>transport capacity rate ratio</u>: the capacity of blood and water to transport oxygen or carbon dioxide,

for oxygen $$\frac{cw}{cb} = \frac{\dot{V}_G\ \alpha w, o_2\ (Pinsp - Pexp)}{\dot{Q}\ \alpha b, o_2\ (Paff - Peff)}*$$

(2) transfer factor: the relative ability of the gill to transfer oxygen per unit tension gradient. It is determined by the area available for exchange, and by the diffusion distance between blood and water (Randall, Holeton, and Stevens, 1967).

*(from Randall, Holeton and Stevens)

(2) transfer factor: (cont'd)

$$\text{for oxygen } To_2 = \frac{\dot{V}o_2}{\Delta Po_2} = \frac{\dot{V}o_2}{\frac{1}{2}(Pinsp + Pexp) - \frac{1}{2}(Peff + Paff)}$$

as the transfer factor increases effectiveness of transfer across the gills will also increase.

(3) number of transfer units: the ratio between the capacity of the gills to transfer oxygen (related to gill area (A) and a coefficient (d) expressing the distances and diffusion characteristics of oxygen in the two media and across the epithelium) and the transport capacity rate of the blood ($\dot{Q}^{\alpha}b$) or water ($\dot{V}_G{}^{\alpha}w$), for oxygen this is:

$$\text{transfer units} = \frac{Ad}{C_b} \text{ or } \frac{Ad}{C_w}$$

(4) flow arrangement: effectiveness will be related to the nature of the flow through the exchanger which can be (a) counter current, (b) cocurrent, or (c) multicapillary.

Reported values of effectiveness in rainbow trout and dogfish are given in Table 7. For both species effectiveness in oxygenating blood is very high (95 to 100% and 79% respectively). Effectiveness in relation to carbon dioxide removal from the blood is somewhat lower (40 to 60% and 40% respectively). Effectiveness of oxygen

removal from the water and carbon dioxide uptake by the water is very low for trout (Table 7). Low values for E_b, CO_2 in both species reflect the low circulating levels of carbon dioxide in the blood under normal conditions. The extremely low values for E_w, CO_2 and E_w, O_2 in trout can be related to its high ventilation volume, and low utilization as compared to dogfish and the large capacity of water for carbon dioxide.

The concept of effectiveness differs from utilization in two ways: it takes conditions in the blood, as well as in the water, into account in assessing the performance of the gills. Effectiveness is the ratio of the rates of gas transfer, while utilization is simply a measure of the quantity of oxygen removed from the water. Randall, Holeton and Stevens (1967) have described the relationship between utilization and effectiveness of oxygen removal from the water (E_w, O_2) as follows,

$$E_w, O_2 = \%U \frac{P_{insp}}{P_{insp} - P_{aff}}$$

E_w, O_2 and %U are closely related and become equal when P_{aff}, O_2 (venous oxygen tension) reduces to zero. The difference between E_w, O_2 and %U increases with P_{aff}, O_2, and it is possible to have a high E_w, O_2 and a low %U under certain conditions.

The transport capacity rate ratio incorporates the carrying capacities and flow rates of blood and water into the concept

Table 7. The effectiveness of gas exchange in rainbow trout* (Salmo gairdneri) and dogfish** (Scyliorhinus stellaris).

		Trout		Dogfish
		4 - 8°C	15°C	
O_2 uptake by blood	E_b,O_2	100	95	79
CO_2 removal from blood	E_b,CO_2	60	40 - 60	41
O_2 removal from water	E_w,O_2	11	30	66
CO_2 uptake by water	E_w,CO_2	4	6	43
utilization	%U	10	55	62

* Randall, Holeton, and Stevens (1967)

**Piiper, and Baumgarten-Schumann (1968)

of effectiveness. For this reason it is a more useful term for waterbreathers than the $\dot{V}_{G/\dot{Q}}$ ratio normally used in mammalian studies. The transport capacity rate ratio for resting rainbow trout is between I and 5 (Randall, Holeton and Stevens, I967; Cameron and Davis, 1970; Davis and Cameron, 1970). When the capacity rate ratio equals one, a counter current exchange system is superior to a cocurrent system. Theoretical analyses have provided indirect evidence for counter current exchange in teleosts (Piiper and Baumgarten, 1968; Hills and Hughes, 1970; Piiper and Scheid, 1972). The effectiveness of gas exchange will vary with changes in the capacity rate ratio (Hughes and Shelton, 1962; Hughes, 1964). The usefulness of such a ratio is also limited, however, by the involvement of a large number of variables required for its calculation.

Gas exchange is dependent on conditions for the convection of oxygen in water, and its diffusion through the epithelial barrier. The transfer factor (To_2) is useful as it takes these processes into account in assessing the relative ability of the gills to transfer gases.

The transfer factor for oxygen at the gills in resting trout is between 0.0059 and 0.03I0 ml. $min^{-1}kg^{-1}mmHg^{-1}$ (Table 5). That for dogfish is 0.0080 ml. $min^{-1}kg^{-1}mmHg^{-1}$ (Piiper and Baumgarten, 1968).

The transfer factor incorporates the equation for gas transfer (Hughes and Morgan, 1973) into the analysis of gas exchange at the gills,

$$\dot{V}o_2 = \frac{kA\ \Delta Po_2}{t} \quad , \quad \frac{kA}{t} = \frac{\dot{V}o_2}{\Delta Po_2}$$

From the equation it can be predicted that the transfer factor will vary with changes in the area available for exchange (A), and the diffusion distance between blood and water (t). The overall mass transfer coefficient (K) for oxygen transfer from water to blood will vary at different rates of water and blood flow. The transfer factor thus provides a means for determining the conditions for exchange under a variety of circumstances.

That fish can regulate the effective exchange area of the gills, and thereby gill capacity to exchange gases seems probable (Booth, I978). A model involving the recruitment of previously unperfused secondary lamellae now appears most realistic (Randall, I970; Hughes, I972; Steen and Kruyse, I974). Direct evidence for this has been provided by Davis (I972). Using infrared photography he observed increased blood flow to secondary lamellae located towards the distal end of the filaments following a subintestinal venous injection of adrenalin. Booth (I978) injected blood cells marked with a flourescent vital dye into the ventral aorta of rainbow trout (Salmo gairdneri). In this study resting fish under normoxic conditions preferentially perfused secondary lamellae at the basal end of the primary filaments, and primary filaments towards the dorsal end of the gill arches. The observed perfusion pathways accounted for only 58% of the total secondary lamellae present. The role of non-respiratory shunting was insignificant as perfusion of

the central lacunar space was negligible.

If 58% is assumed to be a general resting value for perfusion in rainbow trout some interesting consequences arise. In order to meet increasing oxygen demand fish can presumably increase functional exchange area by approximately 1.7 times. This, however, would not be sufficient to account for the 5 to 6-fold increase in gill oxygen transfer factor observed during exercise in trout (Stevens & Randall, 1967b, Kiceniuk & Jones, 1977). Clearly, other factors must be involved. Kiceniuk and Jones (1977) point out that if preferential perfusion is operating at rest, the mean oxygen tension gradient (ΔPo_2) calculated for the whole gill is likely an overestimate of that which actually exists across perfused lamellae. Using derived values for oxygen tension in water leaving perfused lemallae (67 mm Hg) as opposed to the mixed expired water oxygen tension for the whole gill (102 mm Hg) normally used, the calculated ΔPo_2 for perfused lamellae in resting trout is 25 mm Hg, as compared to 46 mm Hg for the whole gill. Using this value, the ΔPo_2 actually increased 2.2 times during exercise. It also follows that the transfer factor (To_2) for perfused lamellae is higher than that for the whole gill (.022 and .013 ml $min^{-1}kg^{-1}mm\ Hg^{-1}$ respectively), and increases 3.5 times with exercise.

Changes in effective exchange area (x 1.7) and the mean oxygen tension gradient (x 2.2) will therefore account for the increase in the transfer factor (x 3.5). If the calculations based on perfused lamellae are valid, other factors, however, must be involved to achieve the increases in oxygen uptake which have been observed (Stevens & Randall, 1967; Kiceniuk & Jones, 1977). In addition to increases in ventilation volume and cardiac output, Jones and Randall (1978) have suggested the redistribution of blood flow at the secondary lamellae, reduction in the diffusion distance by means of a decrease in the thickness of the boundary layer of water or the mucous coat covering the epithelium may be involved. For example, the flow of water

through the gill sieve appears to be laminar, and the thin boundary layer of water at the secondary lamellar surface constitutes a major impediment (80 to 90%) to diffusion (Hills & Hughes, 1970). It will be obvious, therefore, that reduction in boundary layer thickness, such as presumably occurs with increasing water velocity, would be highly effective in increasing transfer capacity; probably more so than the changes in the water-to-blood diffusion distances which could be obtained by adjustment of the mucous coat.

The term "number of transfer units" (Hughes & Shelton, 1962; Hughes, 1964) is analagous to "transfer factor" in that it takes into account the gas transfer equation, and incorporates conditions for convection in water and diffusion in assessing gill transfer capacity. The problem with this parameter, as Randall (1970b) has pointed out, is that it is difficult to calculate due to the complexity of the terms involved.

In conclusion, it is important to realize that the theoretical analyses described here have been simplified by the nature of the underlying assumptions. These are not necessarily valid under actual conditions for gas exchange. In particular, they ignore non-linearity of the dissociation curves describing the relationship between partial pressure and content for oxygen and carbon dioxide in the blood, and carbon dioxide in the water.

(g) The Effect of Temperature

(i) General considerations

In poikilothermic vertebrates, such as fish, changes in the ambient temperature have dramatic effects on cellular rate functions. Poikilotherms are not totally dependent on temperature, however, and within a defined thermal range they can compensate to varying degrees for changes in temperature via adjustment of oxidative metabolism, and/or changes in their internal milieu (Prosser, 1967). The interaction between these two processes is complex, and depends on rate and magnitude of temperature variation. In general, responses to acute changes in temperature will involve the former, while chronic changes will involve the latter response. When a new physiological steady state is achieved following long term exposure to environments differing in a limited number of well-defined parameters (e.g. temperature) acclimation is said to have occurred (Hill, 1976).

Teleosts exhibit incomplete or partial compensation for temperature (Fig. 10). The chronic response is less thermally-sensitive than is the acute response as indicated by the undershoot in metabolic rate in response to the initial rapid drop in temperature. The subsequent rise in metabolic rate to some intermediate level indicates partial compensation for temperature-induced rate changes with time. The extent to which fish can compensate for temperature determines their thermal tolerance limits, and the level of activity which they are able to sustain within this compatible range.

The dependence of oxidative metabolism on temperature in teleosts is shown in Fig. 11. Metabolic rate is related to temperature in an approximately exponential fashion as indicated by the small degree of nonlinearity of the semilogarithmic plots. This relationship is not surprising, since metabolism represents the sum total of chemical reactions whose rates are only partially compensated for by the acclimatory response to long-term exposures at different

Figure 10. Changes in ventilatory rate and oxygen consumption in gold fish following a decrease in temperature from 27 to 15°C (modified from Heath, 1973).

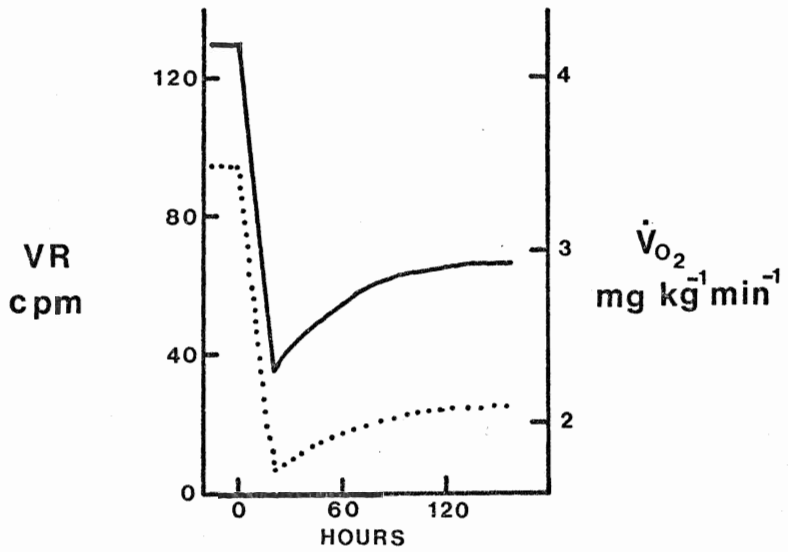
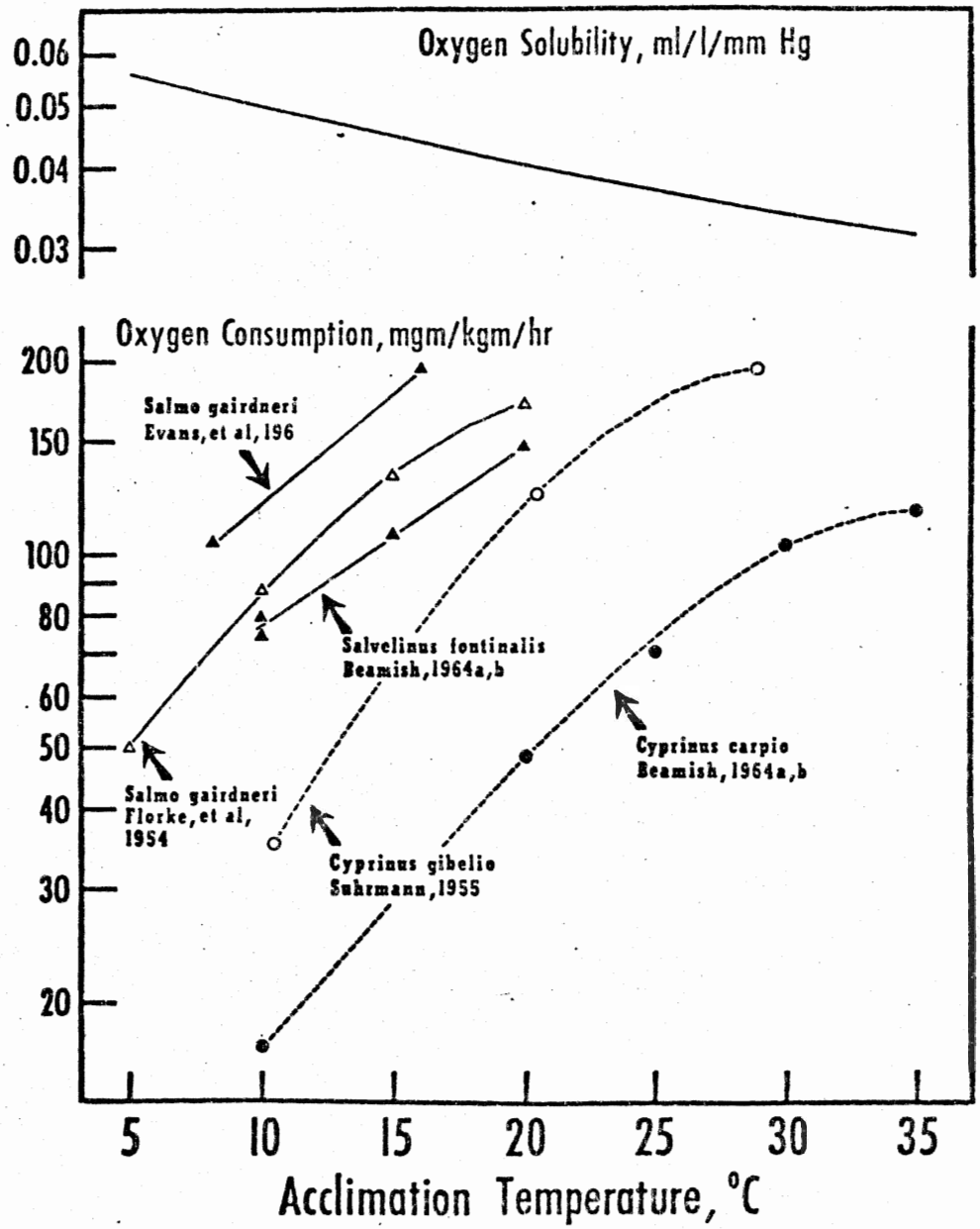

VR
cpm
120
80
40
0
0
60
120
HOURS
4
3
2
$\dot{V}_{O_2}$
mg kg^{-1} min^{-1}
O_2 consumption ($\dot{V}_{O_2}$)
ventilatory rate (VR)

Figure 11. Changes in oxygen availability and oxygen consumption with temperature following thermal acclimation in teleosts (taken with permission from Houston, 1973).


Oxygen Solubility, ml/l/mm Hg
0.06
0.05
0.04
0.03
Oxygen Consumption, mgm/kgm/hr
200
150
100
80
60
50
40
30
20
Salmo gairdneri
Evans, et al, 196
Salvelinus fontinalis
Beamish, 1964a, b
Salmo gairdneri
Florke, et al,
1954
Cyprinus carpio
Beamish, 1964a, b
Cyprinus gibelio
Suhrmann, 1955
5
10
15
20
25
30
35
Acclimation Temperature, °C

temperatures. The steep slope of the semilogarithmic plots indicates that thermal sensitivity is high in teleosts, but decreases as the acclimation temperature increases. The relatively broad thermal range over which teleosts remain active, however, indicates that these fish are able to alter their ventilatory, circulatory, and gas exchange processes so that they can cope with large temperature induced variations in oxygen demand.

(ii) The effect of temperature on the physical characteristics of water.

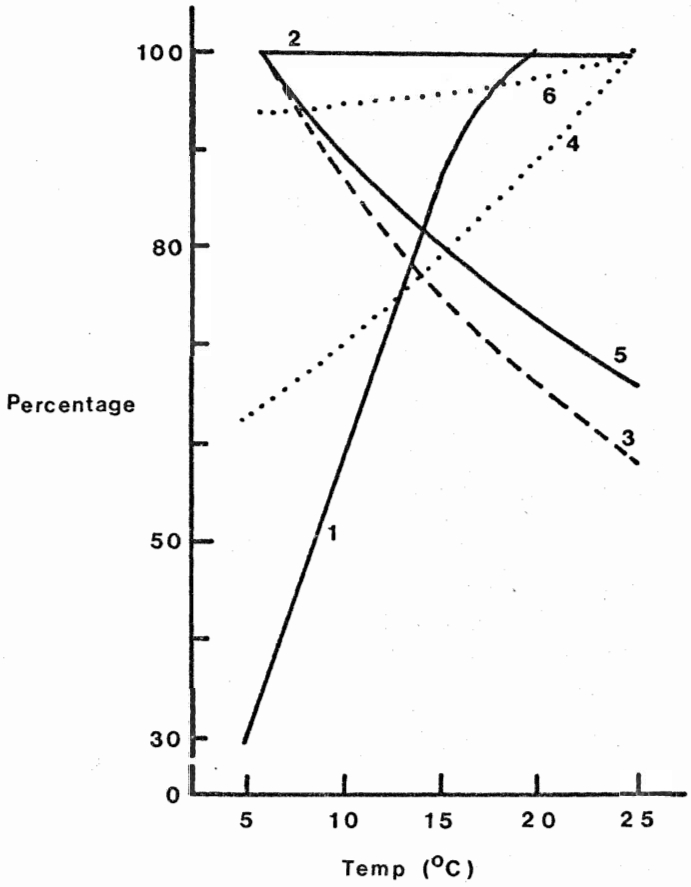
Temperature has a marked effect on the physical characteristics of freshwater (Fig. 12). It is difficult, however, as Hughes and Roberts (1970) have pointed out, to fully understand the overall effect of these temperature-induced changes in water upon gas exchange. Some of these effects are, however, evident. In terms of oxygen transport in water, for example, the lowering of viscosity as temperature increases should reduce gill resistance and thereby ventilatory work. This should compensate to some extent for the increased metabolic work involved in increasing ventilation at higher temperatures. Conditions for exchange at the gills, however, may be adversely affected by an increase in temperature. The large decrease in oxygen solubility ($2 - 3\%^{\circ}C^{-1}$) is partially offset by increase in diffusion rate. The permeation coefficient (D^{1}), given by the product of solubility (α) and diffusion rate (D), however, increases slightly at higher temperatures. On the other hand, the oxygen tension in water remains fairly constant with temperature, and this helps to maintain the oxygen tension gradient (ΔPo_2) across the gill epithelium.

It appears, therefore, that the major effect of temperature lies in a decrease in oxygen solubility (α) which reduces the amount of oxygen available for diffusion across the exchanger. The ability of fish to withstand temperature variation may be dependent on the extent to which ventilation (convective oxygen transport) can be increased to meet the rising oxygen demand before

Figure 12. Graphical representation of the effect of temperature on the physical characteristics of fresh water. Changes in oxygen uptake are also included. (modified from Hughes and Roberts, 1970).

The key to the parameters is as follows:

1. oxygen uptake (ml kg^{-1} hr^{-1})
2. density of water
3. viscosity of water (centipoises)
4. diffusion rate (D, cm^2 $sec.^{-1} \times 10^5$)
5. absorption coefficient (α, ml O_2 ml H_2O^{-1})
6. permeation coefficient ($D^1 = \alpha \times D(\times 10^2)$)


100
80
50
30
0
Percentage
2
6
4
5
3
1
5
10
15
20
25
Temp (°C)

energetic requirements of the branchial musculature become prohibitive.

(iii) Adaptation of the cardiovascular-respiratory complex to changes in temperature.

The ability of fish to adjust gas exchange rates is dependent upon conditions for convection of gases in water and blood, and for their diffusion through the epithelial barriers. This section will deal with the modifiable components of the oxygen transport system in teleosts which could potentially be involved in satisfying temperature-induced increases in oxygen demand. The effect of temperature on the carbon dioxide transport system will not be discussed, as it is considered to be non-rate limiting with respect to temperature.

The types of adjustment available to fish includes the following.

(I) Increased ventilatory flow. This response could compensate for the decreased oxygen-carrying capacity of water with increased temperature.

(2) Increased cardiac output, by transporting more oxygen to the tissues per unit time to meet their increased O_2 requirements.

(3) Increased blood oxygen-carrying capacity, to transport more oxygen per unit blood.

(4) Modification of conditions for gas exchange across the respiratory epithelia to enhance gas transfer.

(iv) Ventilatory changes with temperature.

The response of the ventilatory system to temperature has been studied in several fish species. (Heath, 1973; Roberts, 1973). It is apparent that temperature-dependent changes in ventilation involve adjustments in frequency and stroke volume. The latter response appears to be particularly important, especially during the initial stages of thermal stress.

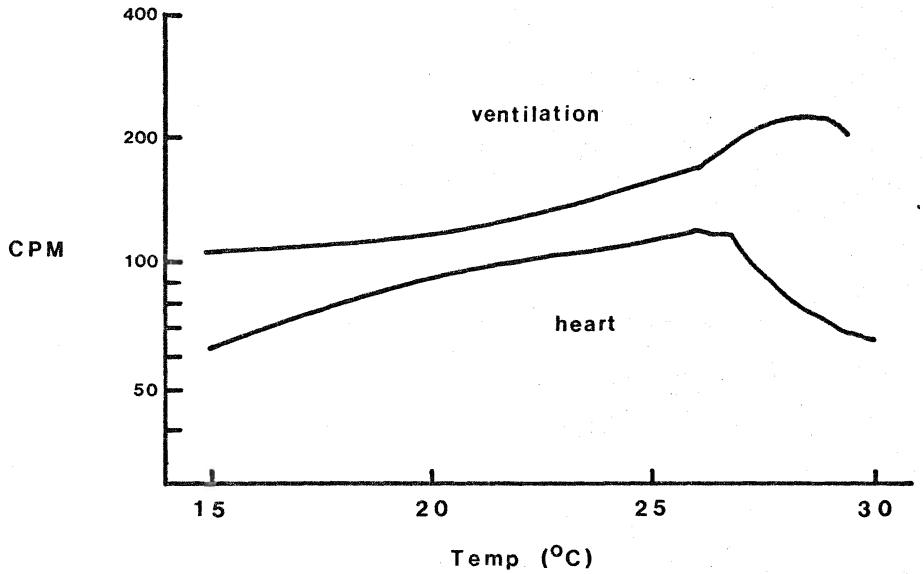
The effect of acute changes in temperature upon the respiratory pumps of lightly-anesthetized rainbow trout has been described by Hughes and Roberts

(1970). In this study ventilatory frequency increased 1.81 times when the water temperature was increased from 16 to 26°C at a rate of 1°C .3 min^{-1} (Fig. 13). Analysis of the amplitudes of pressure waveforms for the respiratory pumps indicated that the initial response to temperature involved significant increases in stroke volume. Heath and Hughes (1973) subsequently exposed unanesthetized rainbow trout to a slower warming rate (1.5°C $.hr^{-1}$) until the lethal temperature was reached (26°C). Ventilatory frequency increased 1.50 times between 15 and 26°C (Fig. 13), but changes in buccal and opercular pressure waveforms were greater than those observed by Hughes and Roberts (1970). The depth of breathing in unanesthetized fish exposed to a slower warming rate continued to increase until temperatures approached the lethal limit. Just prior to death (20 - 26°C) amplitudes dropped sharply and the respiratory pumps became dissynchronous. This was followed by abrupt cessation of all respiratory activity at 26 to 27°C. In this study changes in stroke volume, as indicated by the pressure amplitudes, appeared to be of greater importance than rate changes.

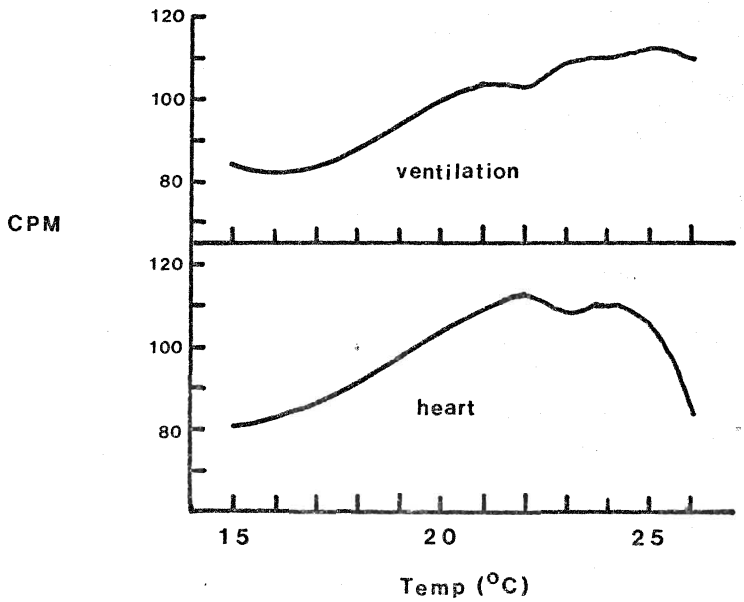
(v) Circulatory changes with temperature.

Relatively little information exists concerning changes in the cardiovascular system with temperature. Any increase in temperature results in increases in cardiac output in intact fish (Randall, 1968). Stevens, Bennion, Randall, and Shelton (1972), for example, have described the effect of an increase in temperature (3°C hr^{-1}) in unrestrained lingcod (Ophiodon elongatus). As the temperature rose from 5 to 15°C, a marked increase in heart rate (x 2.3) was observed, with little change in stroke volume (Fig. 14). Using unanaesthetized rainbow trout Heath and Hughes (1973) observed smaller increases in heart rate (x 1.38) with temperature (1.5°C $.hr^{-1}$) from 15 to 23°C (Fig. 13). Lightly-anaesthetized rainbow trout responded to more rapid warming (1°C .3 min^{-1}) by increasing heart rate some 1.76 times between 15 and 26°C (Fig. 13).

Figure 13. The effect of temperature on ventilatory frequency and heart rate in rainbow trout.(a) modified from Hughes and Roberts, (1970), warming rate $1^{o}C$ 3 min^{-1}. (b) modified from Heath and Hughes, (1973), warming rate $1.5^{o}C$ hr^{-1}.

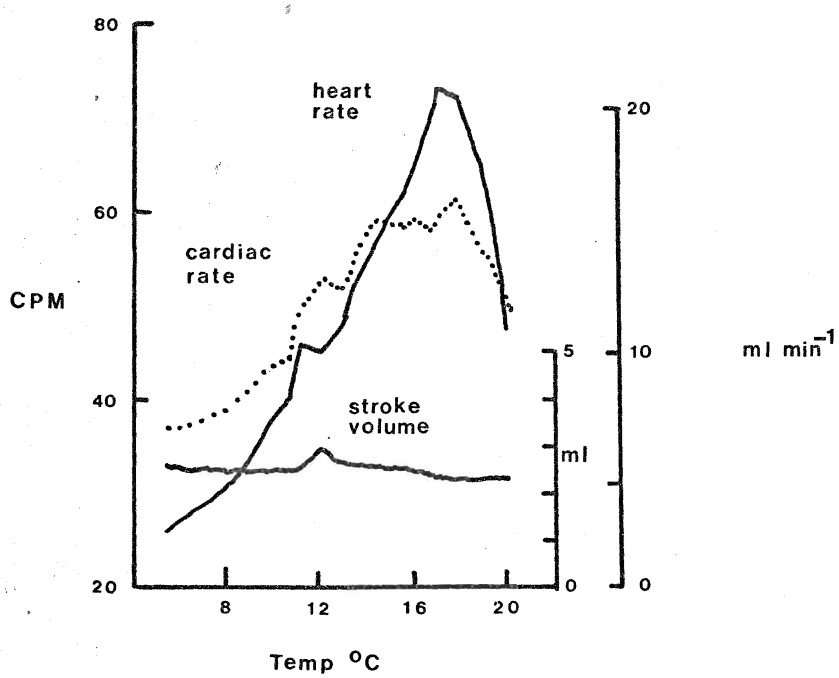

400
200
100
50
CPM
ventilation
heart
15
20
25
30
Temp (°C)

(a)


120
100
80
CPM
ventilation
120
100
80
heart
15
20
25
Temp (°C)

(b)

Figure 14. The effect of an increase in temperature ($3^{o}C\ hr^{-1}$) on heart rate, stroke volume, and cardiac output in unrestrained lingcod (modified from Stevens, Bennion, Randall, and Shelton, 1972).


80
60
40
20
CPM
heart
rate
cardiac
rate
stroke
volume
5
ml
0
20
10
0
ml min⁻¹
8
12
16
20
Temp °C

Changes in cardiac activity with temperature have been attributed to the direct effect of temperature on pacemaker cells of the heart, increasing their intrinsic rate. The work of Bennion (1968; cited by Randall, 1970) who observed increases in heart rate with temperature in the isolated trout heart, for example, supports this view. Cardiac output changed little, however, because of simultaneous decreases in stroke volume. The maintenance of stroke volume in the lingcod, however, indicates that extrinsic factors, such as peripheral resistance changes and the cardiovascular control system are probably involved as well. (Stevens, et al., 1972). Indirect evidence (Davis, 1972; cited by Randall, 1970a) suggests that peripheral resistance decreases as temperature rises. This effect should increase venous return to the heart, tending to maintain stroke volume via the Starling effect despite the negative ionotropic effect of increasing temperature.

Blood pressure in the dorsal and ventral aortae, and the pressure differential across the gills also increases with temperature, (Heath & Hughes, 1973). This response may involve a number of factors including decreases in peripheral resistance, and (or) increases in the resistances of gill vessels. There appears to be no information in the literature regarding the role of catecholamines or neural innervation in regulating cardiac activity in response to temperature.

(vi) Hematological changes with temperature.

Variations in blood oxygen-carrying capacity would seem to be an obvious means of enhancing oxygen uptake with temperature. This type of compensatory response could be realized through changes in the level of hemoglobin in the blood, either by increasing the number of red blood cells present, or the amount of hemoglobin per red cell.

Studies carried out to date (see Houston, Mearow, & Smeda, 1976, for review) suggest, however, that quantitative hematological changes involving

carrying capacity are not of major importance in chronic and acute responses to temperature.

Another type of hematological response which has received attention involves the inherent complexity of the blood oxygen transport system. Hemoglobin in teleost fishes consists of families of electrophoretically-distinct polymorphs (Riggs, 1970) which may differ in their transport characteristics (Binotti, et al., 1971). Consequently, temperature-dependent modifications in the types and amounts of hemoglobins may produce some adaptive advantage to the blood in response to temperature-induced increases in oxygen demand (Houston, Mearow, & Smeda, 1976). The limited number of studies upon hemoglobin heterogeneity in thermally-acclimated species of fish, however, have not provided a definitive answer to this question as yet (Houston, Mearow, & Smeda, 1976; Weber, Wood, & Lomholt, 1976).

(vii) Changes in the conditions for diffusion at the gills with temperature.

There is a paucity of data concerning modifications in gas exchange at the gills with temperature. It seems apparent, however, that conditions for diffusion at the respiratory membranes must also vary with changes in temperature.

Rainbow-trout (Heath & Hughes, 1973) exhibited a slight decline in blood oxygen content and capacity of the dorsal aortic blood with increasing temperature. This was especially notable at temperatures above 20°C, where ventilatory problems also were first observed. Arterio-venous oxygen differences were slightly increased, however, due to simultaneous decreases in venous oxygen content. This fell to zero at 23°C. This response indicates that conditions at the branchial exchanger were such that arterial blood could not be fully saturated at high temperatures.

In view of decreases in arterial and venous oxygen tension, it is possible

that the oxygen tension gradient across the gills (ΔPo_2) increases slightly with temperature. Oxygen tensions in water passing over the gills would be expected to remain high due to the increase in ventilation associated with increasing temperature. Consequently, the ability of the gills to transfer oxygen per unit of tension gradient (i.e., the transfer factor, To_2), may increase with temperature to account for the large increase in oxygen uptake observed (2 - 3 times resting $\dot{V}_{O_2}$).

Although relevant data is lacking, fish may also modify the effective exchange area of the gills via lamellar recruitment in response to temperature. The importance of this factor in increasing the transfer factor (To_2) is unknown. A response of this type, however, would invoke ionoregulatory and osmoregulatory problems, especially at high temperatures when exchange area and rates of water influx and electrolyte efflux would be maximized (Evans, 1969; Maetz, 1972).

In summary, the fish may compensate for changes in temperature by modifying ventilation, perfusion, and conditions for gas exchange at the gills. Very little is known about the relative contribution of each of these components in response to temperature. From the data of Heath and Hughes (1973) changes in ventilation and perfusion would be expected to provide an increase in convective oxygen transport capability of 1.40 to 1.50 times. This increase in itself however, is not sufficient to account for the increase in oxygen uptake (x 2.72) observed. The arterio-venous oxygen difference increased 1.25 times with temperature. This increase in the effective oxygen transport of the blood, combined with that of convective transport (x 2.25) is still insufficient to account for the increased oxygen uptake observed. The extent to which modifications in diffusive transport are involved in response to temperature is not clear. The decline in arterial blood oxygen saturation at high temperatures (20 - 26°C) indicates that the efficiency of

the exchanger is decreased under these conditions. Watters and Smith (1973) noted a similar response to temperature in starry flounder. (Platichthys stellatus). With an increase in temperature between 11 and 20°C, they observed a slight decrease in the arterial blood oxygen saturation, although the arterio-venous oxygen difference remained constant. There was little change in the effectiveness of oxygen uptake by the blood (Eb,O_2), and only a slight decrease in effectiveness of oxygen removal from the water (Ew,O_2). The major effect of elevated temperature on the flounder lay in increasing oxygen uptake, with concurrent increases in water and blood flow rates. That increases in flow rates were closely regulated was indicated by the constancy of the $\dot{V}_G/\dot{Q}$ ratio. In this way the impact of the dead space phenomena, discussed previously, could have been reduced. It would appear that the major rate-limiting factor is the diffusion resistance of the gills in relation to the reduced oxygen levels in the water. This probably accounts for the drop in arterial blood saturation at high temperatures.

(viii) Changes in standard and active metabolism with temperature.

As noted previously, the exact nature of the temperature response will depend on the rate and magnitude of the thermal change. In most instances, however, the type of response will ultimately be dictated by changes in oxygen demand resulting from changes in energy metabolism and activity.

Fry (1957) has distinguished three categories of oxygen consumption; standard, routine, and active. Standard oxygen consumption is that required to maintain basal physiological functions. Routine oxygen consumption is the mean oxygen uptake of resting fish exhibiting spontaneous activity. Active oxygen consumption is that observed under conditions of maximal activity. The difference between active and standard metabolism is defined as the metabolic scope for activity (Fry, 1957). The difference between active and routine metabolism is defined as the routine scope for activity (Beamish, 1964).

"Scope" sets a limitation for activity with variations in temperature. The influence of temperature on the metabolic and routine "scopes for activity" is given in Figs. 15 and 16 respectively. For yearling sockeye salmon standard metabolism increases with temperature, and markedly so above 12°C. Metabolic scope for activity in salmon, and routine scope for activity in brook trout both show temperature optima around 15°C. Brett (1964) proposed that it was the low level of oxygen in the water which limited metabolic scope above 15°C. It is evident from this study that the cost of delivering oxygen to the tissues increases with temperature, but as Randall (1970b) suggested, other factors such as the resistance to diffusion at the gills must be involved in rate-limiting effects at high temperatures. Dickson and Kramer (1971) have also described a similar temperature effect on metabolic scope in rainbow trout, finding the maximum scope for activity to be between 15 and 20°C.

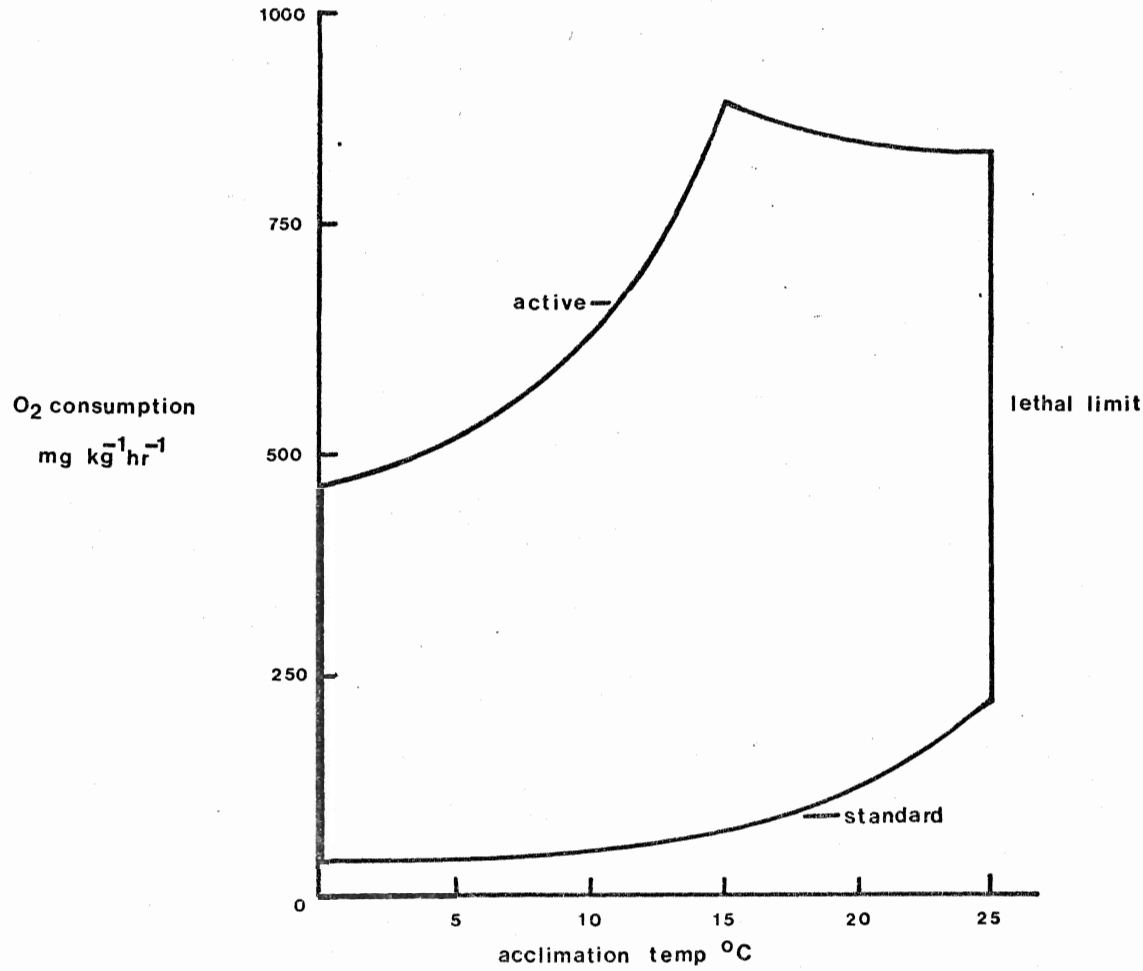
(ix) Possible causes for ventilatory and cardiac failure at high temperatures.

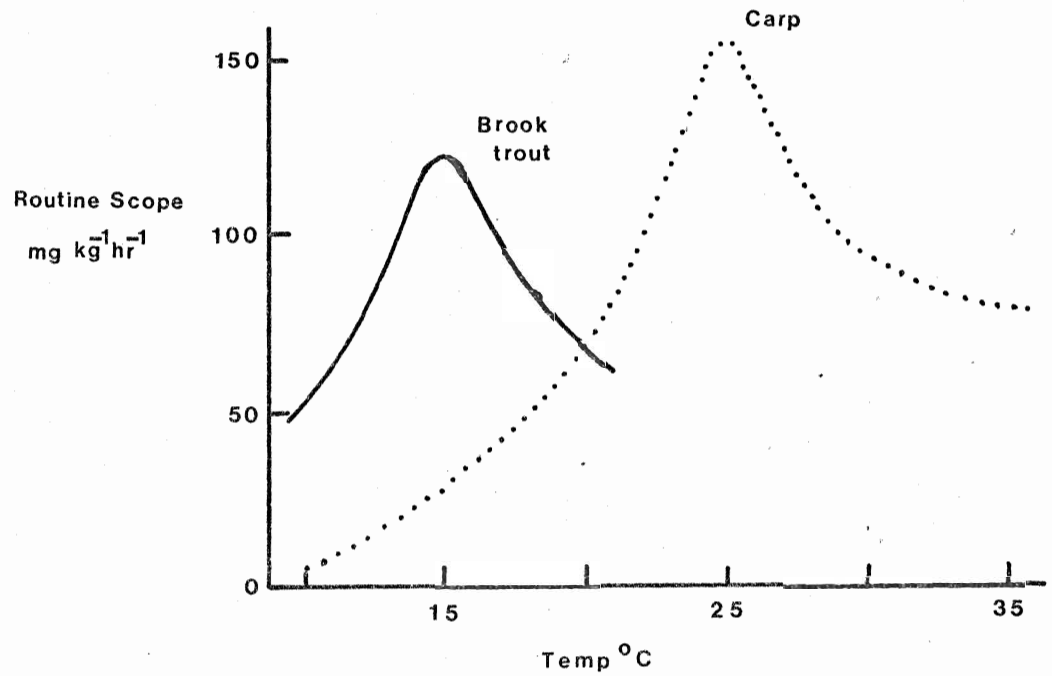
Clearly the metabolic cost of increasing water and blood flow with temperature will eventually become prohibitive, whereby the fish is caught in a situation of diminishing returns. In rainbow trout exposed to acute lethal thermal stress (Heath & Hughes, 1973) ventilation and oxygen consumption levelled off at high temperatures (23 to 25°C), while heart rate decreased until death occurred at 26°C. The actual cause of thermal death is not well understood (Fry, 1957; Roberts, 1973), but it is felt to involve a failure in the overall integration of physiological systems rather than any one component.

It is generally believed that the nervous system is most susceptible to temperature; there is some evidence implicating central nervous system control of respiration in respiratory failure at high temperatures. For example, Roberts (1973) monitored Po_2 levels in the brains of lightly anesthetized, unrestrained rainbow trout subjected to rapid warming (1°C .3 min^{-1}). As

Figure 15. The effect of acclimation temperature on the metabolic scope for activity in yearling sockeye salmon (modified from Brett, 1964).

Figure 16. The effect of acclimation temperature on the routine scope for activity (modified from Beamish, 1964).


1000
750
500
250
0
active
standard
lethal limit
O_2 consumption
mg $kg^{-1}hr^{-1}$
5
10
15
20
25
acclimation temp °C


150
100
50
0
Carp
Brook
trout
Routine Scope
mg $kg^{-1}hr^{-1}$
15
25
35
Temp °C

temperature increased brain Po_2 levels decreased. At temperatures of 25°C and above normal ventilation was apparently unable to maintain adequate oxygen levels in the brain to sustain normal nervous activity. Respiratory aberrations were noted at these temperatures, and were followed by abrupt cessation of ventilation, and death at 27°C. Similar responses have been described by Hughes and Roberts (1970) and Heath and Hughes (1973) for rainbow trout. In these studies dissynchronization developed between the respiratory pumps, and frequent double reversals occurred as temperature approached 25°C. An abrupt decrease in the pressure amplitudes of the buccal and opercular pumps and ventilatory frequency preceeded death at 26 to 27°C. The consensus of opinion is that the respiratory pumps become increasingly more inefficient as temperature increases until Po_2 levels in the brain drop to such low levels that respiratory nervous activity ceases. Further support for this theory comes from the fact that artificial ventilation of the gills with water can sustain fish at and above the normal lethal temperature by partial restoration of brain Po_2 levels (Roberts, 1973).

Bradycardia develops in fish exposed to acute thermal stress at high temperatures (Roberts, 1968, 1970; Hughes & Roberts, 1970; Heath & Hughes, 1973), and in response to hypoxia (Randall & Smith, 1967; Holeton & Randall, 1967a). The efferent pathway for this response is via vagal cholinergic fibres since bilateral vagotomy, or pretreatment with atropine partially abolishes it (Roberts, 1973). It has been proposed that the cardio-inhibition observed under thermal stress is also a response to either internal or external hypoxia (Hughes & Saunders, 1970; Heath & Hughes, 1973; Roberts, 1973). At high temperatures, inadequacies in oxygen transport result in the oxygen in the blood becoming depleted which evokes bradycardia. The decrease in heart rate continues until death finally occurs.

MATERIALS AND METHOD

1. Origin and Maintenance of Fish

Rainbow trout (16 month) were obtained from a local hatchery, Goosen's Trout Farm in Otterville, Ontario. All stocks were held in continuous flow, fiberglass Frigid Unit MT-700 tanks of 500 litre capacity, with a water turnover rate of 2 to 3 times per day. Dechlorinated St. Catharines tap water (total hardness - 145 mg l^{-1} as $CaCO_3$, total alkalinity - 100 mg l^{-1} as $CaCO_3$, pH 7.5 - 8.0 was used throughout the study. Dissolved oxygen levels were 80% of saturation or greater. The fish were fed twice daily, ad libitum, with a pelleted trout ration (Purina trout chow). The tank water was continuously filtered through polyurethane foam, and excess food and feces were removed daily.

Fish were starved in the holding tank for 24 hours prior to sampling in an effort to minimize the amount of metabolic waste introduced into the respirometer during experiments.

All stocks were initially held at 8 - 10°C for 1 week, and exposed to a 12-12, light-dark photoperiod. They were then brought to the desired acclimation temperature at a rate of 1C° day^{-1} and held at this temperature for a minimum period of 4 weeks before use. Final acclimation regimes employed are shown in Fig. 17. Three groups of fish were acclimated to static temperatures of 2°, 10°, and 18°C, and one to a diurnal sinusoidal temperature cycle (midpoint - 10°C, amplitude $\pm$ 4°C). Temperature control was effected by "Minnow Cool" circulator/coolers working concurrently with 1000 watt stainless steel heating coils which were regulated by platinum resistance thermometers, temperature controllers accurate to $\pm$ 0.1°C. Diurnal temperature cycles were imposed by means of a low frequency sine wave generator. All temperatures were recorded by means of a Fisher certified thermometer.

The temperatures at which tests were carried out are as shown in Fig. 18.

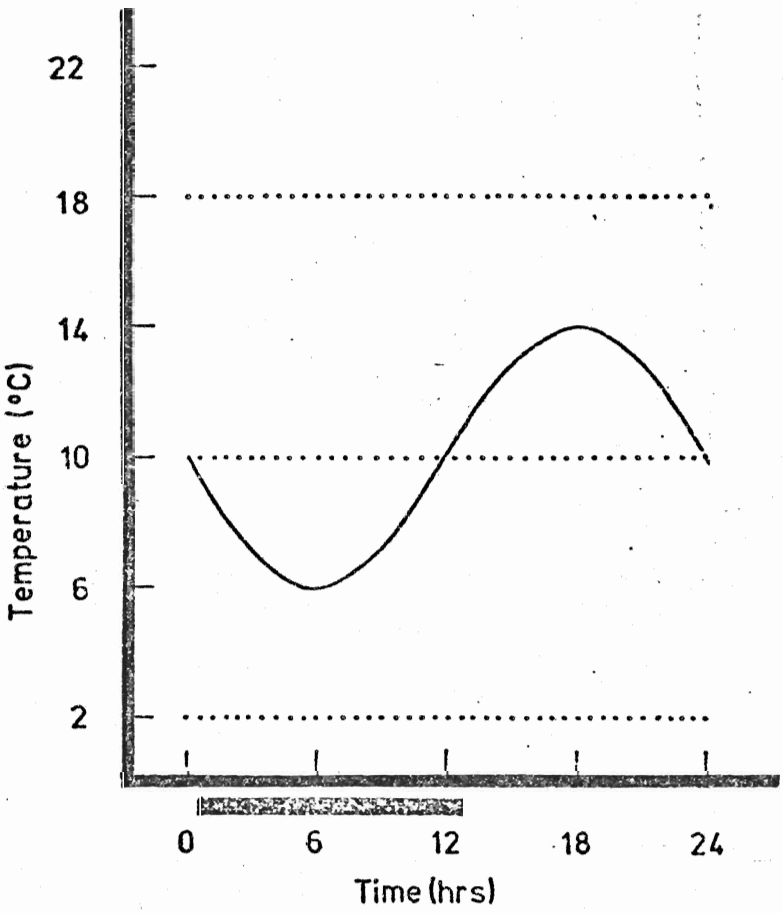

22
18
14
10
6
2
Temperature (°C)
0
6
12
18
24
Time (hrs)

Figure 17. Diagram showing the acclimation regime used in the study.

.....statically acclimated fish

_____ cyclically acclimated fish

The horizontal black bar represents the dark period of the 12/12 L/D photoperiod.

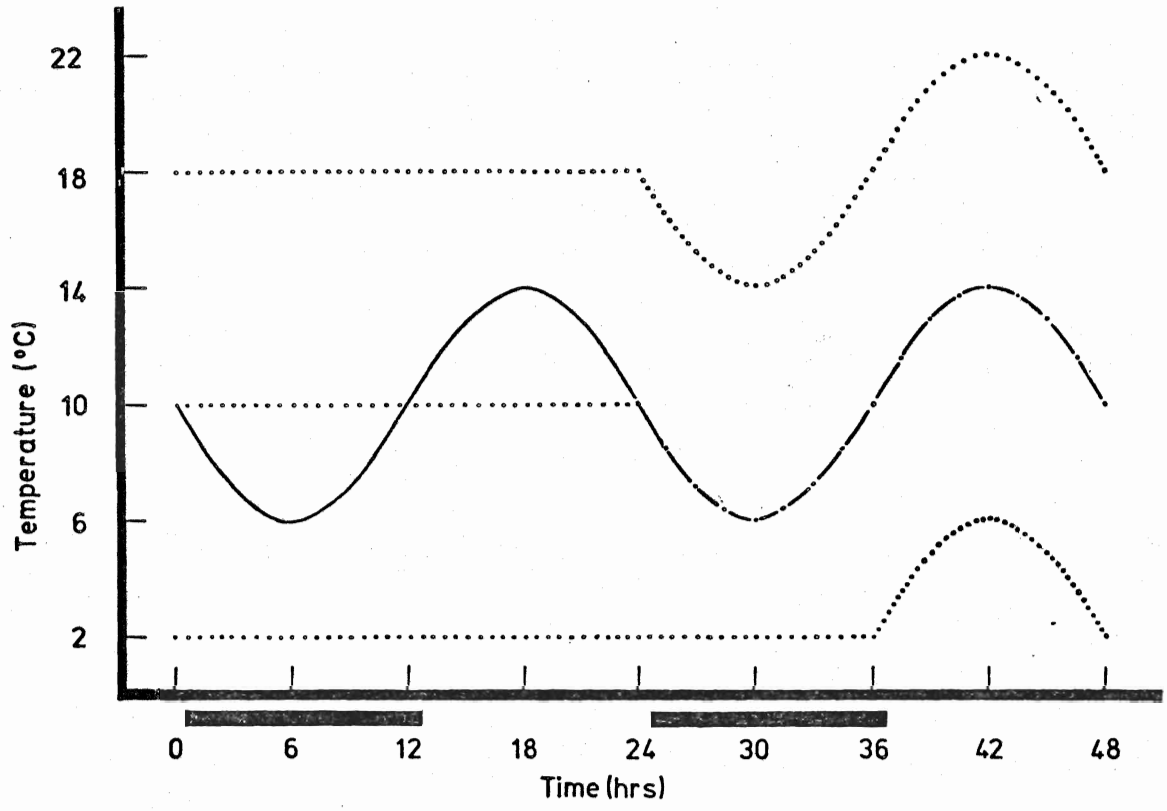

22
18
14
10
6
2
Temperature (°C)
0
6
12
18
24
30
36
42
48
Time (hrs)

Figure 18. Diagram showing the experimental tests used in the study.

..... for fish acclimated to static temperatures.

_____ for fish acclimated to cyclic temperatures.

The horizontal black bars represent the dark period of the 12/12, L/D photoperiod.

Following a 36 hour post preparative recovery period, the three groups of fish acclimated to static temperatures were tested for one day at acclimation temperature, and then for an additional day while exposed to a diurnal temperature cycle. (midpoint-acclimation temperature, amplitude $\pm$ 4°C). It should be noted that the 2°C- fish were exposed only to the upper portion of the cycle. The cyclically-acclimated fish were exposed to the same diurnal temperature cycle for the entire 48 hour sampling period.

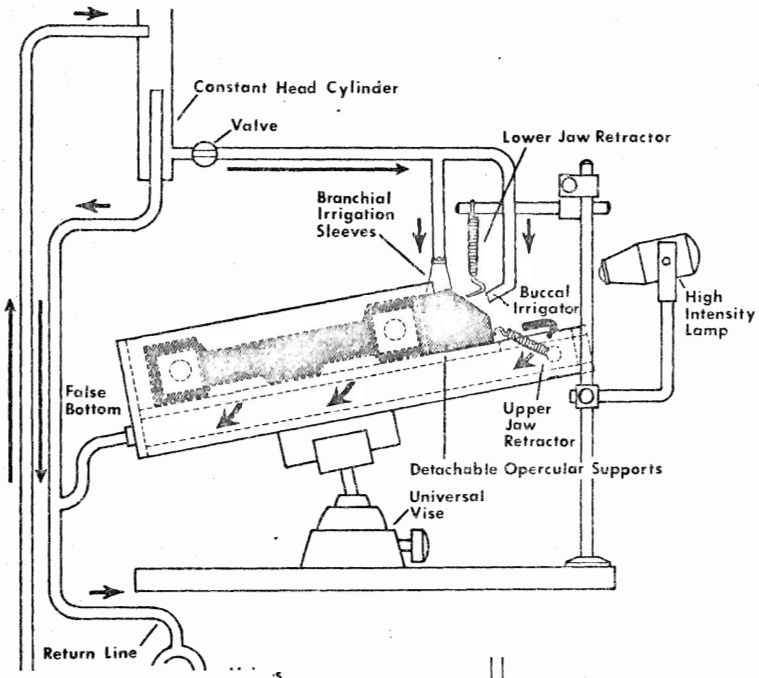
2. Preparation of Specimens.

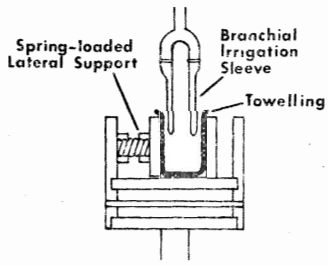
Test fish were anesthetized in a 5 litre glass aquarium containing freshly prepared tricaine methane sulphonate solution (100 mg l^{-1}) at the acclimation temperature. After anesthetization they were transferred to an operating assembly (Fig. 19). This was equipped with a circulating pump and nozzles to irrigate the gills and mouth with aerated water containing fresh anesthetic (10 mg l^{-1}). Each fish was then fitted with three electrocardiograph subdermal pin electrodes (E2B) and with buccal and cleithral cannulae (P.E. 160) as shown in Fig. 20. The duration of the operation, including anesthetization, was 25 to 30 minutes. The fish were then transferred to plexiglass restraining trays and loosely secured by means of sutures through the tail and dorsal fin. The trays were then inserted in cylindrical respirometer chambers (42.5 cm x 11.0 cm), and immersed in the upper tank of the respirometer. All of these maneuvers were carried out with the fish submerged in the water to enhance recovery.

Care was taken to insulate the test organisms from audiovisual disturbances. The upper chamber of the respirometer was lined with flat mat paper similar in color to the holding tanks (dull green), and fitted with a styrofoam lid. This insured that the fish would not be disturbed by movement around the respirometer.

Figure 19. Operating system for fishes (with permission of A.H. Houston).

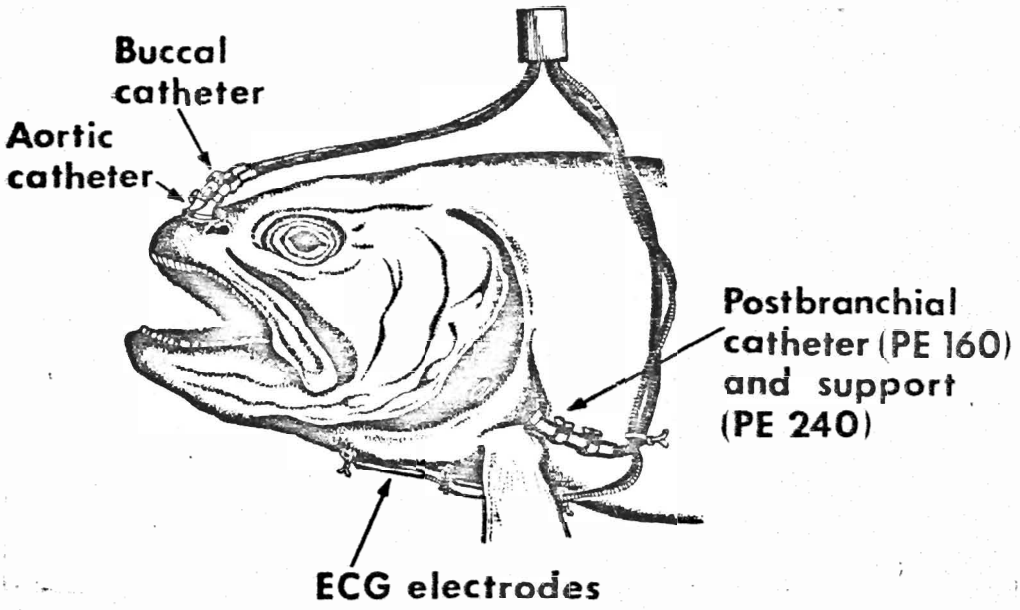
OPERATING SYSTEM FOR FISHES

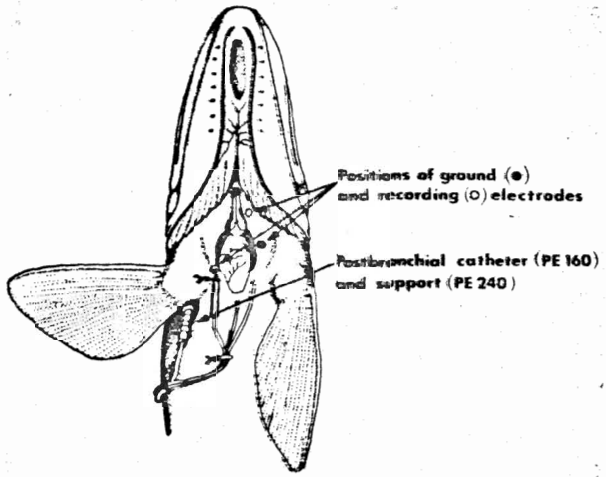




FRONT END OF OPERATING BOARD

Figure 20. Diagram showing the placement of cannulae and ECG electrodes in fish. (a) lateral view of head. (b) ventral view showing placement of ECG electrodes in the chest cavity in relation to the position of the heart (with permission of A.H. Houston).


Buccal catheter
Aortic catheter
Postbranchial catheter (PE 160) and support (PE 240)
ECG electrodes


Positions of ground (●) and recording (○) electrodes
Postbranchial catheter (PE 160) and support (PE 240)

3. Determination of Respiratory-Cardiovascular Activity.

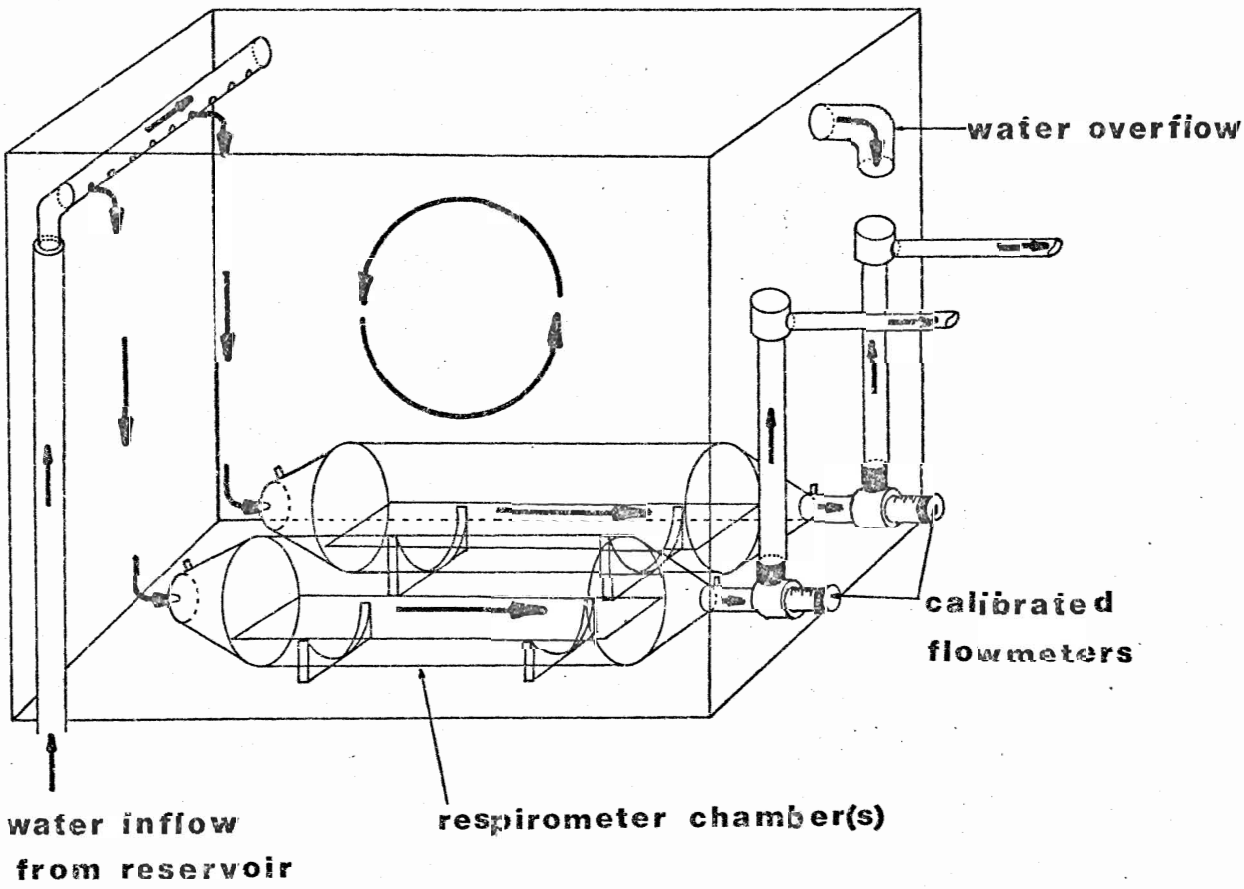
The respirometer (Fig. 21) was a modified version of the constant-pressure head design developed by Beamish and Mookherjii (1964). It was of 1/2" plexiglass construction, and consisted of an upper experimental tank which held two respirometer chambers, and a lower reservoir containing the temperature controlling units, water aeration, and filtration devices. Water flow through the respirometer chambers was regulated by means of Gilson calibrated #3 flowmeters equipped with micrometer valves, and having a rated accuracy of $\pm$ 2%. Water flow through each chamber was maintained at 250 mls min^{-1} in all experiments.

Estimates of oxygen consumption ($\dot{V}_{O_2}$) utilization (%U), ventilatory flow ($\dot{V}_G$) and stroke volume (V_{S_V}) were obtained using the Fick principle, in conjunction with the cannulation techniques described above. The mean oxygen content (mg l^{-1}) of water samples drawn from water entering and leaving the chambers, and from water entering and leaving the gills were determined using the phenyl-arsine oxide procedure.

Buccal and opercular pressures were recorded with Statham P23BC pressure transducers, linked through Grass 7P/B low level DC preamplifiers to a Grass model 7B polygraph. A Grass model TM-1 transducer mixer was also employed in certain instances. Equivalent lengths of polyethylene tubing (PE 160) were used on opposite sides of each transducer. Turning of the appropriate stopcocks allowed recording of zero, and ventilatory pressures. Catheters were attached to the transducers by Luer stub adaptors (18 gauge) and spring-loaded stopcocks (BDMS10, BDMS02).

The experimental procedure was as follows: Ten fish were used in each of the four tests. Fish were cannulated, and fitted with ECG electrodes at 8 - 9 a.m., enclosed in their chambers and placed in the respirometer assembly. Each animal was allowed to recover for 36 hours prior to recording. Twenty-four hours after preparation cannulae and ECG electrodes were connected to the recording system, which was then calibrated. The fish were then left undis-

Fig. 21. Constant-head respirometer used in the study.


water overflow
calibrated
flowmeters
water inflow
from reservoir
respirometer chamber(s)

turbed for 12 hours prior to the first sample, which was taken at ∿ 8 p.m. Recording of buccal pressure, cleithral pressure, and ECG was followed by determinations of chamber flow rates, water temperature, inflow, outflow, inspired and expired water oxygen levels. Records were taken every 6 hours from the onset of sampling. All experiments continued for 48 hours.

4. Analysis of Data.

Typical recordings are shown in Fig. 22. For detailed analysis of pressure waveforms the following methods were used. The total amplitude including both + and -ve phases of buccal and opercular pressures was measured directly. The mean area beneath the buccal and opercular pressure waveforms was measured with a planimeter. Simultaneous recordings of buccal and opercular pressures were used to derive the area differential pressure. The differential pressure record (Fig. 23) was divided into 3 phases (a), (b), and (c). During phase (a) the negative pressure in the opercular cavity exceeds that in the buccal cavity. During phase (b) the positive pressure in the buccal cavity exceeds that in the opercular cavity. Phase (c) is a reversal pressure, when the sign of the pressure reverses from + to -ve. The areas of these 3 components were measured so as to indicate the relative contribution of each respiratory phase to the flow of water over the gills. The area (Arbitrary units) measured under any given pressure waveform was multiplied by the ventilation frequency and divided by time (60 sec.) to calculate area mean pressure. This manipulation takes into account the changes in ventilatory frequency which accompany changes in temperature. The area differential pressure was multiplied by the ventilatory frequency to give the relative minute volume (RMV), a differential pressure equivalent of the minute volume (Hughes & Roberts, 1970). The area mean differential pressure was obtained by dividing the relative minute volume, by time expressed in the same units as for differential area measurements.

Figure 22. Simultaneous recordings of opercular and buccal pressures. The ECG trace is shown below. The buccal and opercular waveforms are shown transposed on one another indicating the opercular and buccal components of the differential pressure.

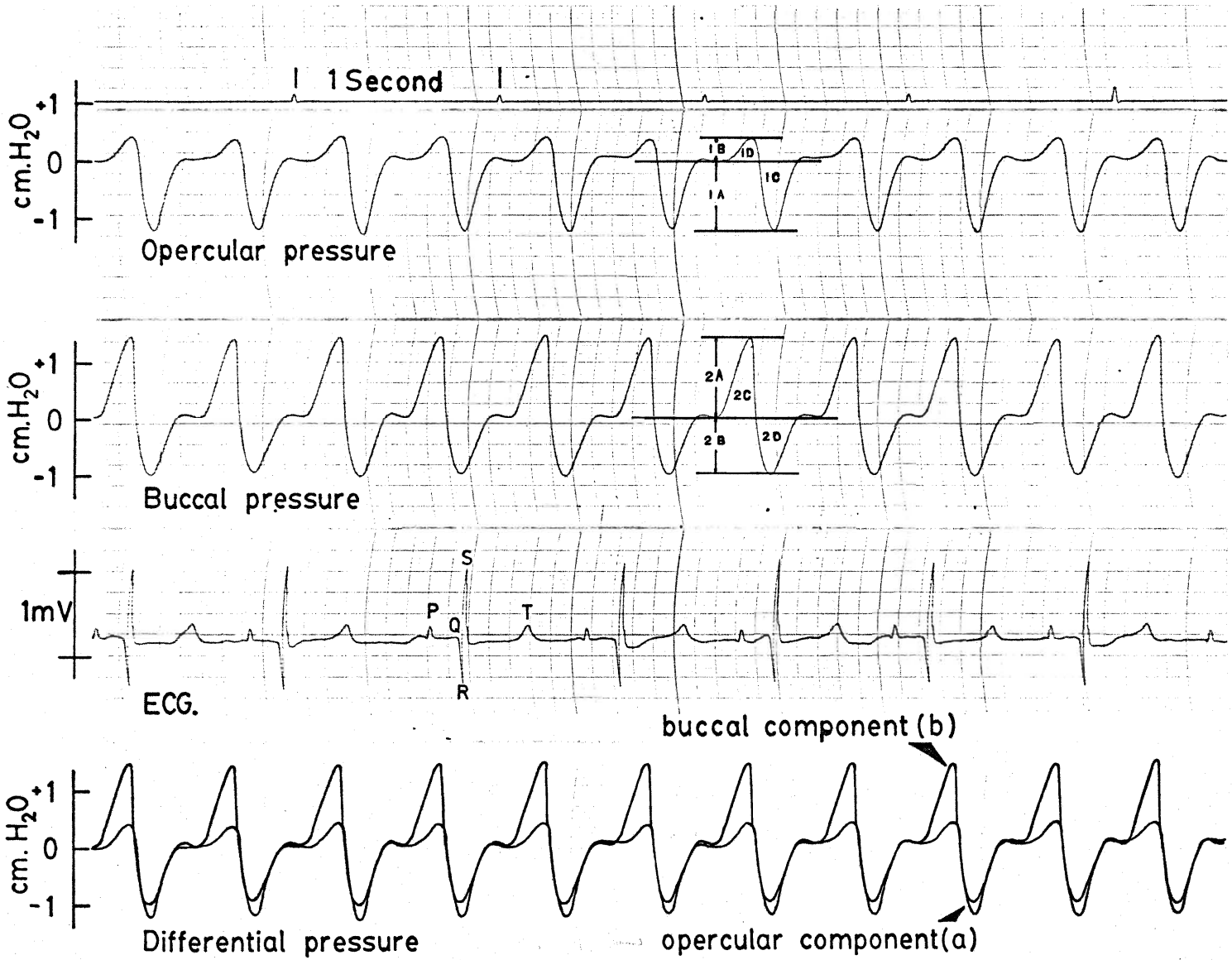
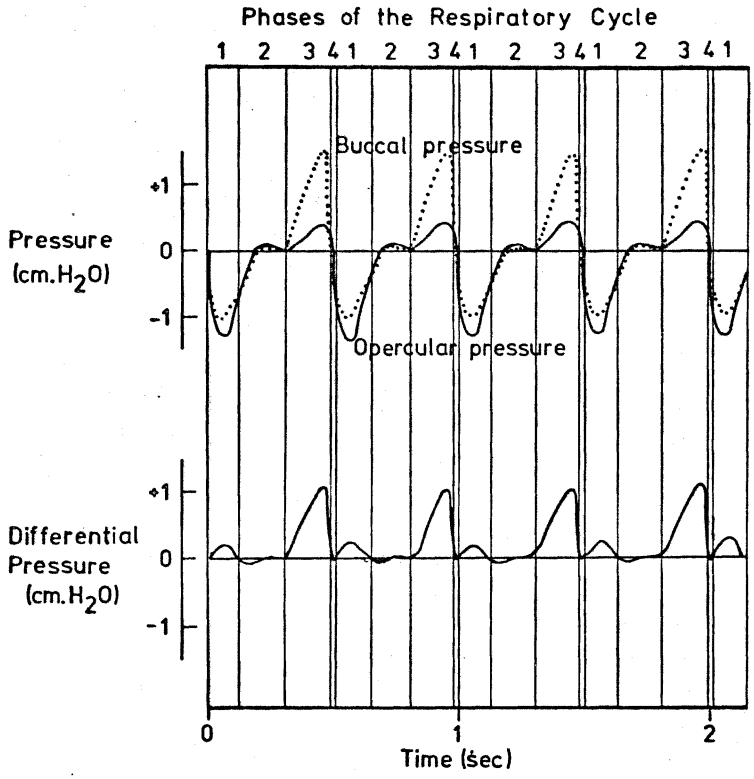

1 Second
cm.H2O
+1
0
-1
1B
1D
1C
1A
Opercular pressure
2A
2C
2B
2D
Buccal pressure
1mV
S
P
Q
T
R
ECG.
buccal component (b)
Differential pressure
opercular component(a)

Figure 23. Simultaneous recordings of opercular and buccal pressures shown transposed upon one another. The derived differential pressure (Hughes and Shelton, 1958) is included below. The differential pressure is positive when the pressure in the buccal cavity exceeds that in the opercular cavity. Zero pressure is equivalent to the ambient pressure of the water surrounding the fish. The major phases of the respiratory cycle are included above: (1) in which the opercular suction pump is predominant; (3) when the buccal force pump is predominant; (2) and (4) transition periods when the sign of the differential pressure may reverse.


Phases of the Respiratory Cycle
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1
Buccal pressure
Opercular pressure
Pressure (cm.H2O)
+1
0
-1
Differential Pressure (cm.H2O)
+1
0
-1
0
1
2
Time (sec)

The timecourse of the P wave, QRS complex, and T wave, and the amplitude of the QRS complex as well as frequency were obtained from EKG traces. A more thorough treatment of the procedures and formulae used in obtaining the various ventilatory-cardiovascular parameters employed in the study is included in the Appendix.

5. Statistical Analysis.

Statistical analyses were carried out using a Wang 2200 computer. Means, standard error, and 95% confidence intervals were used for descriptive purposes (Alders & Rossler, 1968). The student t- test and one-way analysis of variance were used to determine the significance of temperature-induced changes in each parameter.

6. Explanation of Symbols used.

$\dot{V}_{O_2}$ oxygen uptake per unit time in mg Kg^{-1} hr^{-1}.

%U, percentage of oxygen removed from inspired water.

RMV, relative minute volume

$\dot{V}_G$, ventilation volume per unit time in ml min^{-1}.

Vsv, ventilatory stroke volume per breathing cycle in ml.

GR, gill resistance, ratio

MDP, mean differential pressure per unit time in arbitrary units.

VR, ventilation rate per min.

CR, cardiac rate per min.

CoR, coughing rate per min.

For a thorough treatment of formulae used in calculating the above see appendix C and D.

RESULTS

RESULTS

The effects of temperature on ventilatory-cardiovascular function will be considered under three principal headings. The effects of acclimation will be discussed insofar as they are apparent in the three groups acclimated to constant temperature conditions. The effects of short-term cyclical change in temperature will be considered in specimens previously acclimated to constant conditions. The effects of longer-term acclimation to diurnally-cycling temperatures will be considered in relation to those of the first two.

All data are recorded in Appendices (Appendix: Tables 2 to 29), and has been summarized in this section in Tables 8 through 12, and Figs. 24 through 36. In the case of Figures the same format has been used throughout, with recorded values being presented as the means $\pm$ 95% confidence intervals (vertical bars). Open bars represent fish acclimated to static temperatures (2°, 10°, 18°C), and closed vertical bars fish acclimated to a diurnal temperature cycle (midpoint 10°C, amplitude $\pm$ 4C°). The closed horizontal bars represent the dark period of the 12/12 LD photoperiod regime employed.

Table 8 in this section contains Q_{10} values for the changes in cardiovascular and respiratory parameters observed within groups of thermally-acclimated rainbow trout during exposure to an initial temperature cycle. Appendix: Table 1 contains examples of the analysis of variance, for selected samples of the measured parameters, used to ascertain the significance (at the 5% level) of observed differences.

At this point it is convenient to note some general features of the cardiovascular-respiratory responses observed for all parameters measured. (1) There were no significant diurnal changes which could be attributed to photoperiod in any of the cardiovascular-respiratory parameters recorded within groups of rainbow trout acclimated to constant temperature.

(2) The effects of temperature decreased with increasing temperature both within

Table 8. Q_{10} values and thermal coefficients for cardiovascular and respiratory parameters recorded from thermally acclimated rainbow trout.

Acclimation temp.	A. static 2°C		B. static 10°C				C. static 18°C				D. diurnal temp. cycle (amp. 8°C, mdp. 10°C)**			
Time (hours)	36-42	42-48	24-30	30-36	36-42	42-48	24-30	30-36	36-42	42-48	0-6	6-12	12-18	18-24
Temperature range (C°)	2-6	6-2	10-6	6-10	10-14	14-10	18-14	14-18	18-22	22-18	24-30	30-36	36-42	42-48
Parameter											10-6	6-10	10-14	14-10
$\dot{V}_{C_2}$	4.56	3.78	3.54	3.12	2.98	2.44	2.21	2.09	2.17	1.99	3.35	2.61	2.91	2.13
* % U	2.00	1.90	2.40	2.15	1.96	2.05	1.50	1.21	1.47	1.09	1.98	1.59	1.85	1.42
RMV	5.69	3.59	4.93	3.92	3.37	3.56	3.30	3.62	2.33	3.03	5.56	3.43	6.95	4.30
$\dot{V}_G$	3.06	2.23	2.35	2.21	2.42	2.37	1.97	2.05	2.41	2.18	2.57	2.07	2.57	2.02
* Vsv	1.77	1.28	1.63	1.49	1.61	1.61	1.29	1.36	1.63	1.73	1.40	1.26	1.40	1.23
* Gill resistance	1.68	1.39	2.18	1.86	1.49	1.57	1.66	1.83	0.94	1.37	2.33	1.40	3.10	1.95
* Max. buc. press.	3.62	2.66	8.89	8.89	2.74	2.91	2.34	1.91	2.29	2.63	3.48	2.50	5.04	4.38
* Max. buc. area	3.59	2.15	8.00	6.15	4.53	3.48	2.79	2.41	1.95	2.13	3.80	3.13	3.85	3.72
* Min. buc. press.	3.41	3.41	12.5	12.5	4.46	4.46	5.35	4.71	2.69	3.13	11.32	7.42	14.1	11.1
* Min. buc. area	3.72	2.80	15.6	16.6	4.81	2.17	5.98	6.06	2.67	2.46	5.22	5.17	7.11	8.39
* Max. operc.press.	1.93	2.25	16.9	8.92	9.88	9.88	1.06	1.87	1.31	2.20	2.23	1.98	2.29	2.73
* Max. operc.area	2.86	2.21	9.88	6.58	6.53	8.92	-.--	1.70	1.74	2.90	1.54	1.54	1.08	1.54
* Min. operc.press.	2.26	4.63	6.29	5.06	10.3	13.1	3.48	4.64	1.30	1.69	6.03	6.36	4.92	5.17
* Min. operc.area.	3.06	3.88	9.19	7.41	4.24	7.53	4.65	7.08	2.14	3.36	7.22	5.04	6.82	6.92
* Area mean diff. pressure	5.89	3.54	4.91	3.88	3.40	3.52	3.30	3.63	2.37	3.09	5.67	3.41	7.00	4.36
* Cperc. comp. a	5.66	2.35	4.44	4.00	2.43	8.11	4.23	6.34	1.62	2.58	8.27	3.75	9.19	5.89
* Buccal comp. b	4.74	6.84	5.16	3.92	4.67	3.31	4.01	3.20	1.92	2.28	8.19	4.42	7.85	5.65
* Reversal comp. c	3.24	2.55	-.--	-.--	-.--	-.--	18.1	11.8	3.04	3.31	-.--	-.--	-.--	-.--
Ventilation rate	1.53	1.82	1.50	1.44	1.61	1.49	1.53	1.52	1.47	1.27	1.81	1.70	1.81	1.68
Cardiac rate	2.03	2.11	1.93	1.86	1.94	1.97	2.03	1.89	0.96	1.07	2.44	1.93	1.91	1.54
* Cardiac to ventilation rate : ratio	1.23	1.19	1.22	1.21	1.27	1.38	1.27	1.22	0.65	0.83	1.35	1.17	1.00	0.92
* P-Q interval ***	1.89	1.89	1.54	1.54	1.69	1.69	1.52	1.52	1.64	1.64	2.22	1.45	3.33	2.50
* Q-S interval ***	-.--	-.--	-.--	-.--	-.--	-.--	-.--	-.--	-.--	-.--	1.64	1.45	1.56	1.56
* S-T interval ***	2.22	2.32	1.85	1.64	2.17	2.27	1.92	2.08	1.35	1.35	2.33	1.89	2.27	2.33

* values recorded are thermal coefficients since rate changes are not involved (see Appendix E).

** values recorded are averages of actual values calculated over the 48 hour sampling period.

*** values recorded are reciprocals of observed intervals (see Appendix E).

and between acclimation groups as indicated by the Q_{10} values in Table 8. Q_{10} values were generally greater at lower temperatures both within acclimation groups in response to short term cyclical changes in temperature, and between groups in response to increased acclimation temperature and the imposed temperature cycle.

In terms of this study, therefore, Q_{10} values for response to imposed temperature cycles would be expected to decrease as acclimation temperature increased (i.e., 2°C fish display greater overall Q_{10}'s than 10°C fish.) This was, in fact, generally true (Table 8). In addition, within acclimation groups, the Q_{10} values for the lower portion of the cycle would be expected to be greater than those for the upper portion. This, however, was not always the case (Table 8).

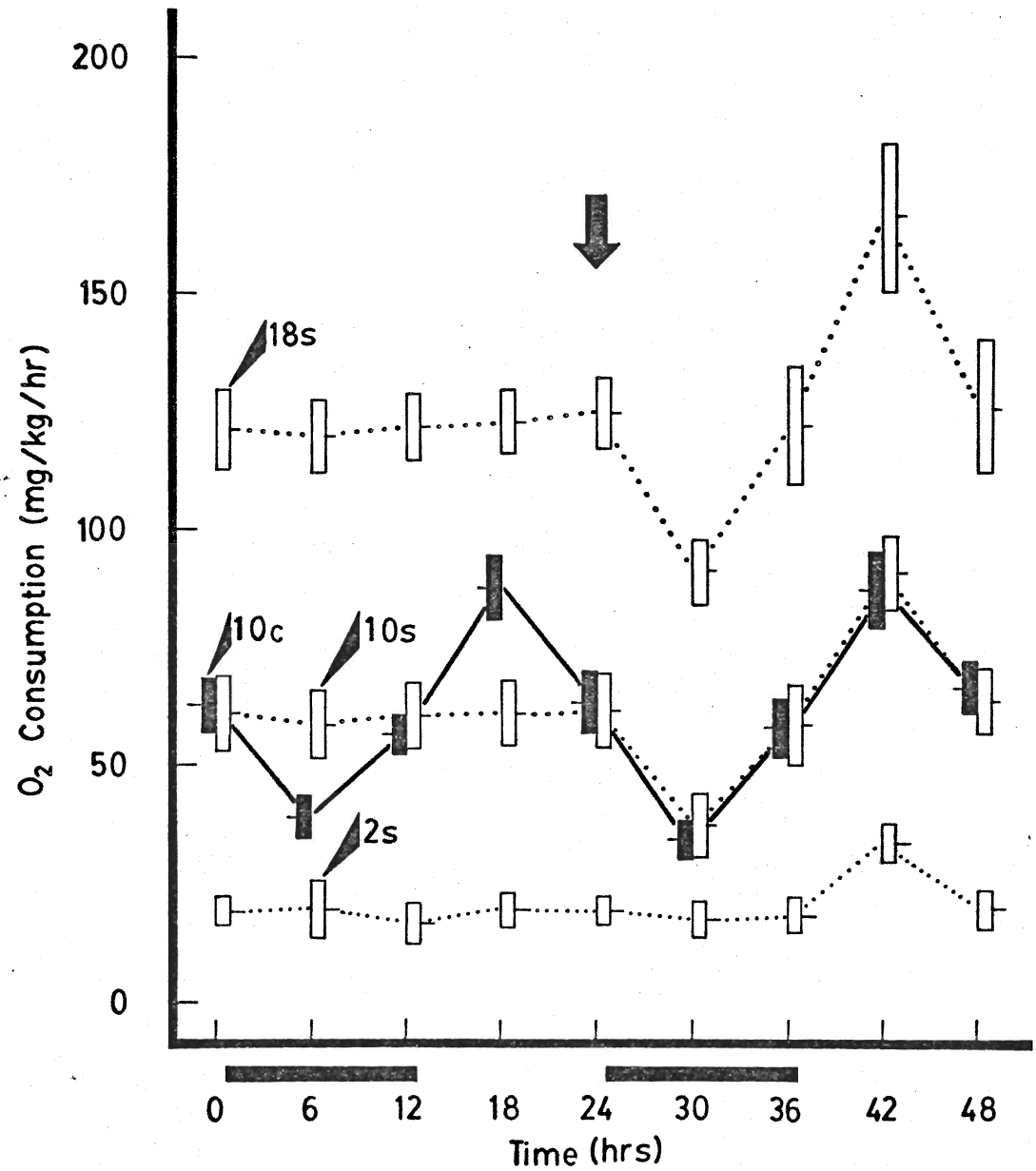
1. Oxygen consumption.

Values recorded for oxygen consumption ($\dot{V}_{O_2}$) (Appendix: Table 2) are summarized in Fig. 24. $\dot{V}_{O_2}$ increased with temperature by some 6.5 times between 2°C and 18°C. Increase in $\dot{V}_{O_2}$ with temperature, however, decreased with increased acclimation temperature, being 3.2 times between 2° and 10°C, and 2.0 times between 10° and 18°C.

Marked changes in $\dot{V}_{O_2}$ were observed in statically acclimated fish (10° and 18°C acclimation) in response to the imposed diurnal temperature cycle. The average Q_{10} values for the cyclic changes were 2.95 and 2.15 for trout acclimated to 10° and 18°C respectively. Fish acclimated to the diurnal temperature cycle (midpoint 10°C, amplitude $\pm$ 4C°) exhibited a marked cyclical response $\dot{V}_{O_2}$ (average Q_{10} - 2.95) which did not differ significantly from that observed for the statically acclimated fish at 10°C.

In both static (10° and 18°C) and cyclically-acclimated trout there was a greater response to the lower portion of the cycle (-4 C°) than the upper portion (+4 C°) as indicated by higher Q_{10} values in each case (Table 8).

Figure 24. Plot showing the effect of temperature on oxygen consumption ($\dot{V}_{O_2}$, mg kg^{-1} hr^{-1}). Vertical bars above and below the mean values represent $\pm$ the 95% confidence interval of the mean. The open vertical bars represent values for fish acclimated to a static temperature (2s - acclimated to 2°C, 10s - acclimated to 10°C, 18s - acclimated to 18°C.) The closed vertical bars represent values for fish acclimated to a diurnal temperature cycle (10c). The closed horizontal bars represent the dark period of the 12/12 LD photoperiod regime used. The arrow indicates the onset of the diurnal temperature cycle for the statically acclimated fish. Note that the fish acclimated to 2°C were only subjected to the upper portion of the cycle (36 to 48 hours).


200
150
100
50
0
O_2 Consumption (mg/kg/hr)
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

2. Utilization

Values recorded for utilization (%U) (Appendix: Table 3) are summarized in Fig. 25. Utilization increased with temperature by some 3.0 times between 2°and 18°C. The magnitude of the change in %U also decreased with increased acclimation temperature, being 2.2 times between 2° and 10°C, and 1.4 times between 10° and 18°C. Moderate changes in %U were observed in fish statically acclimated to 10°C in response to the imposed diurnal temperature cycle. The average Q_{10} value for these cyclic changes ($\pm$ 20 to 30% of midpoint values) was 1.98 for 10°C fish. Rainbow trout acclimated to cycling temperatures exhibited a similar response in %U (Q_{10} - 1.87) which did not differ significantly from that of the 10°C statically-acclimated fish. For fish acclimated to 18°C, however, only small cyclic changes in %U were observed for the lower portion of the temperature cycle (Q_{10} - 1.42), while values for the upper portion of the cycle were not significantly different from those at the midpoint or acclimation temperature.

In the case of trout statically acclimated to 10°C and acclimated to the diurnal temperature cycle there was a greater response in %U to the lower portion of the cycle (- 4C°) than the upper portion (+4C°) as indicated by the higher Q_{10} values in the former case (Table 8).

3. Minute Volume

Values recorded for minute volume ($\dot{V}_G$, branchial water flow) (Appendix: Table 5) are summarized in Fig. 26. $\dot{V}_G$ increased with temperature by some 3.8 times between 2°and 18°C. Change in $\dot{V}_G$ was fairly constant with increases in acclimation temperature, being 2.0 times between 2° and 10°C, and 1.9 times between 10° and 18°C.

Marked cyclical changes were observed in statically-acclimated trout (10° and 18°C) in response to the imposed cycle. The average Q_{10} values for the cyclic changes were 2.34 and 2.15 for 10° and 18°C trout respectively.

Figure 25. Plot showing the effect of temperature on percent utilization (%U). For explanation of symbols used, see text for figure 24.

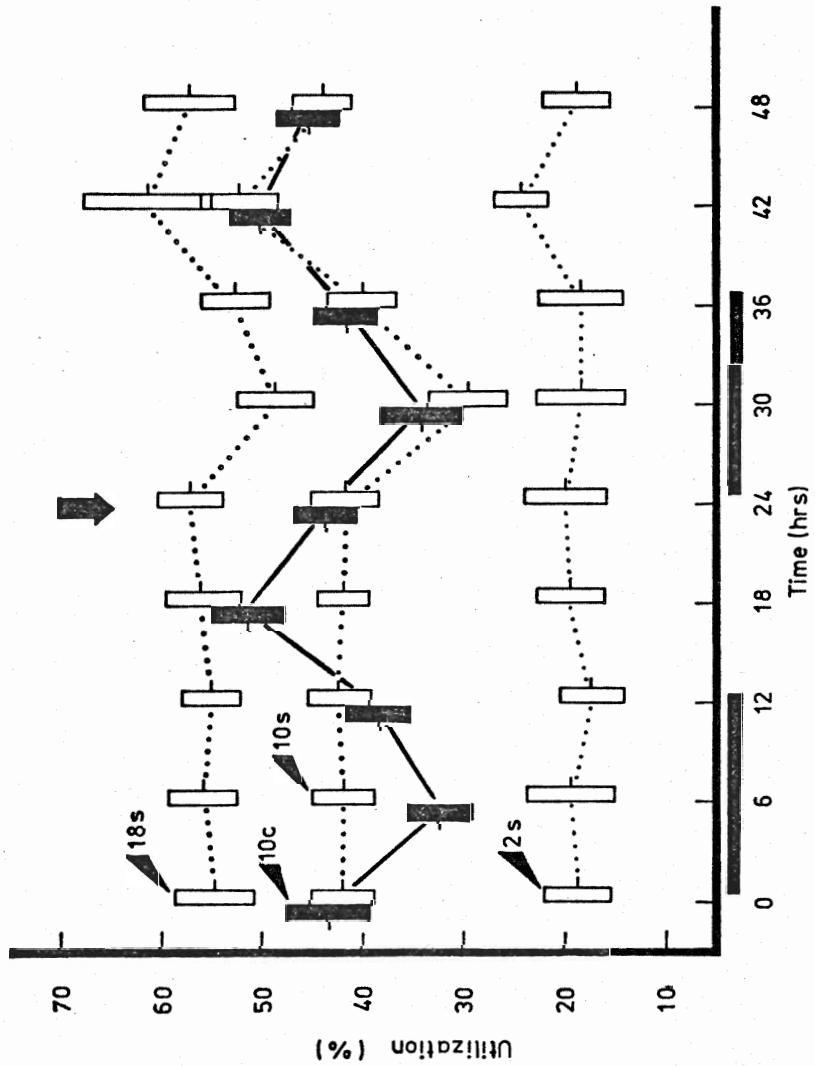
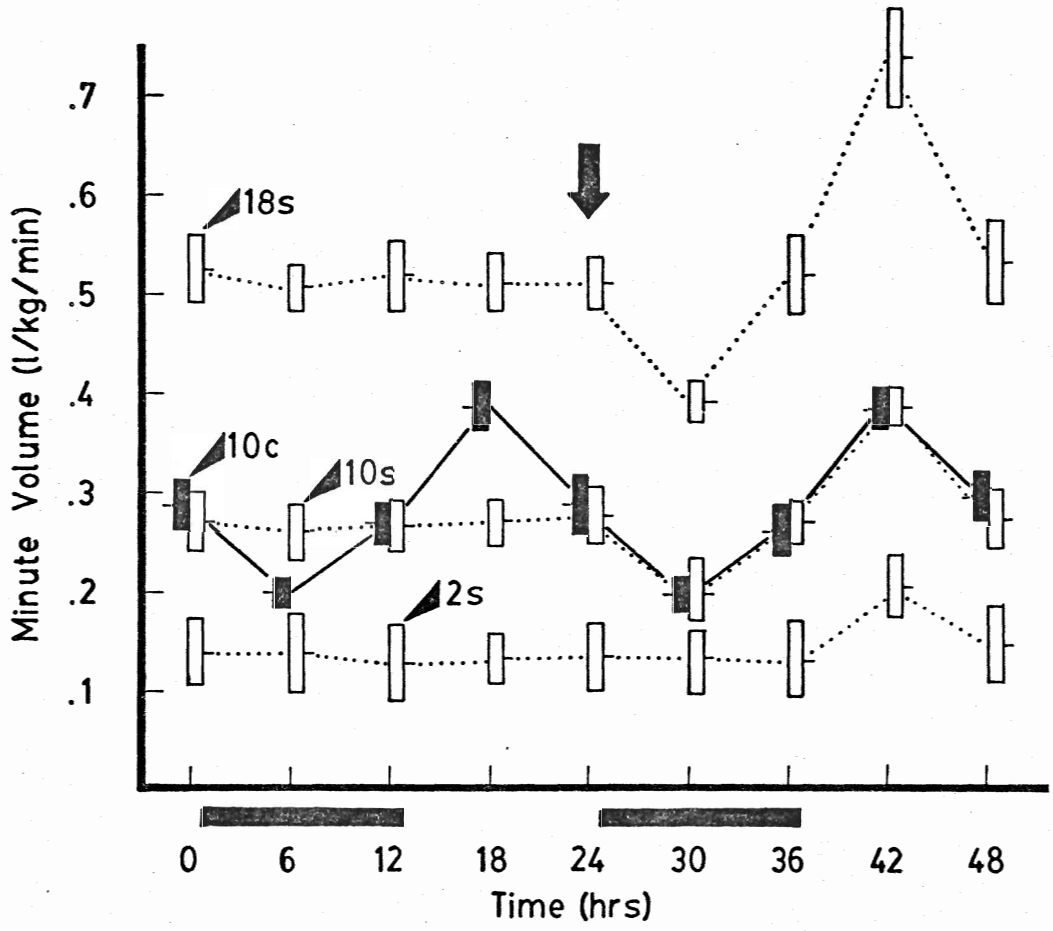

Utilization (%)
70
60
50
40
30
20
10
0
6
12
18
24
30
36
42
48
Time (hrs)
18s
10c
10s
2s

Figure 26. Plot showing the effect of temperature on minute volume ($\dot{V}_G$, l kg^{-1} min^{-1}). For explanations of symbols used, see text for figure 24.


.7
.6
.5
.4
.3
.2
.1
Minute Volume (l/kg/min)
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

Fish acclimated to the diurnal temperature cycle also exhibited a marked cyclic response in $\dot{V}_G$ (average Q_{10} - 2.31), which did not differ significantly from that observed for fish acclimated to 10°C.

For 18 C- acclimated fish the upper portion of the temperature cycle (+4C°) had a greater effect on $\dot{V}_G$ than the lower portion (-4C°). For 10°C-acclimated fish and cyclically-acclimated fish, however, both portions of the cycle appeared to have an equal effect on $\dot{V}_G$ as indicated by the Q_{10}'s for rate changes above and below the midpoint temperature (Table 8).

4. Ventilatory Rate

Values recorded for ventilatory rate (VR) (Appendix: Table 6) are summarized in Fig. 27. Ventilatory rate increased with temperature by some 2.2 times between 2° and 18°C. Increase in VR was fairly constant with increased acclimation temperature being 1.5 times between 2° and 10°C, and 10° and 18°C respectively.

Moderate cyclical changes in ventilatory rate were observed in statically acclimated rainbow trout (10° and 18°C). The average Q_{10} values for the cyclic changes were 1.51 and 1.45 for 10° and 18°C trout respectively. Fish acclimated to the diurnal temperature cycle also exhibited a moderate cyclic response in VR (Q_{10} - 1.75) which differed from that of the 10°C only at the highest point of the cycle.

The cycled fish exhibited a slightly greater increase in ventilatory rate (10 cycles min^{-1} 9%) in response to the peak of the temperature cycle.

There did not appear to be any differences between the response of trout within any acclimatory group to either the upper or lower portion of the temperature cycle as indicated by the component Q_{10} values (Table 8).

5. Cardiac Rate

Values recorded for cardiac rate (CR) (Appendix: Table 7) are summarized in Fig. 28. Cardiac rate increased with temperature by some 2.8 times between

Figure 27. Plot showing the effect of temperature on ventilatory rate (VR, cycles min^{-1}). For explanation of symbols used, see text for Figure 24.

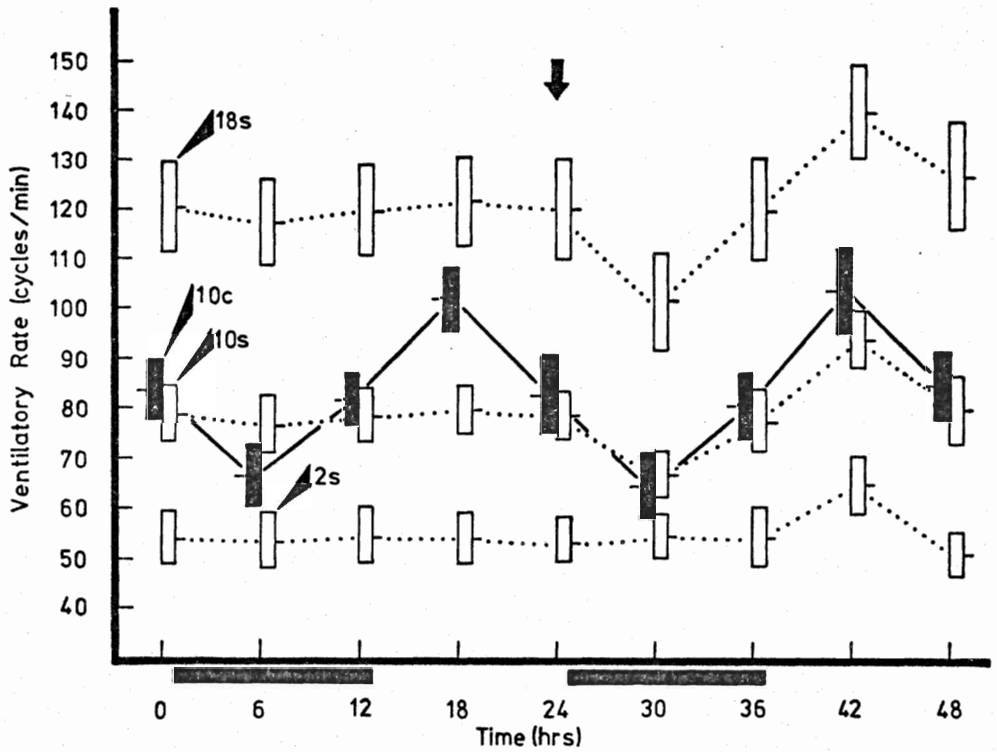
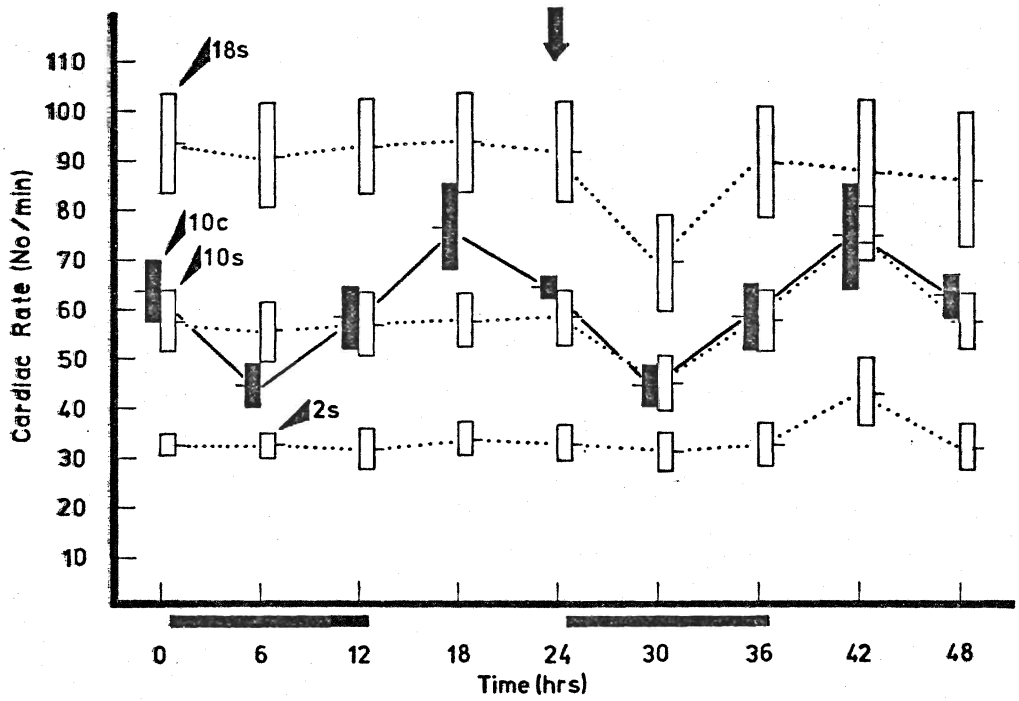

Ventilatory Rate (cycles/min)
150
140
130
120
110
100
90
80
70
60
50
40
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

Figure 28. Plot showing the effect of temperature on cardiac rate (CR, No. min^{-1}). For explanation of symbols used, see text for Figure 24.


18s
10c
10s
2s
Cardiac Rate (No/min)
110
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
42
48
Time (hrs)

2° and 18°C. The magnitude of the change in CR decreased slightly with increased acclimation temperature, being 1.8 times between 2° and 10°C, and 1.6 times between 10° and 18°C.

Moderate changes in cardiac rate were observed in response to the imposed diurnal temperature cycle for fish acclimated to 10°C (average Q_{10} - 1.93). Fish acclimated to the diurnal temperature cycle also exhibited a moderate cyclic response in cardiac rate (average Q_{10} - 1.96) which was slightly different from that of the 10°C fish. For cycled fish the cardiac rate at 24 and 48 hours (same point in cycle) was slightly greater than that observed at 48 hours in 10°C fish. Fish acclimated to 18°C, however, did not increase cardiac rate in response to the upper portion of the temperature cycle (Fig. 28). 18°C fish did, however, exhibit a moderate cyclic response in CR to the lower portion of the temperature cycle (average Q_{10} - 1.96). It should be noted that the response of 18°C fish to the high point of the cycle (22°C) showed considerable individual variation. Depending on the individual, cardiac rate either increased, decreased, or remained constant relative to the midpoint value (18°C).

For trout acclimated to 10°C no differences were observed in the response of cardiac rate to either the upper or lower portions of the temperature cycle. Cyclically acclimated fish, however, showed a greater response to the lower portion of the cycle. This effect results from the slightly higher mean cardiac rates observed at 24 and 48 hours in cycled fish (Fig. 28).

6. Stroke Volume

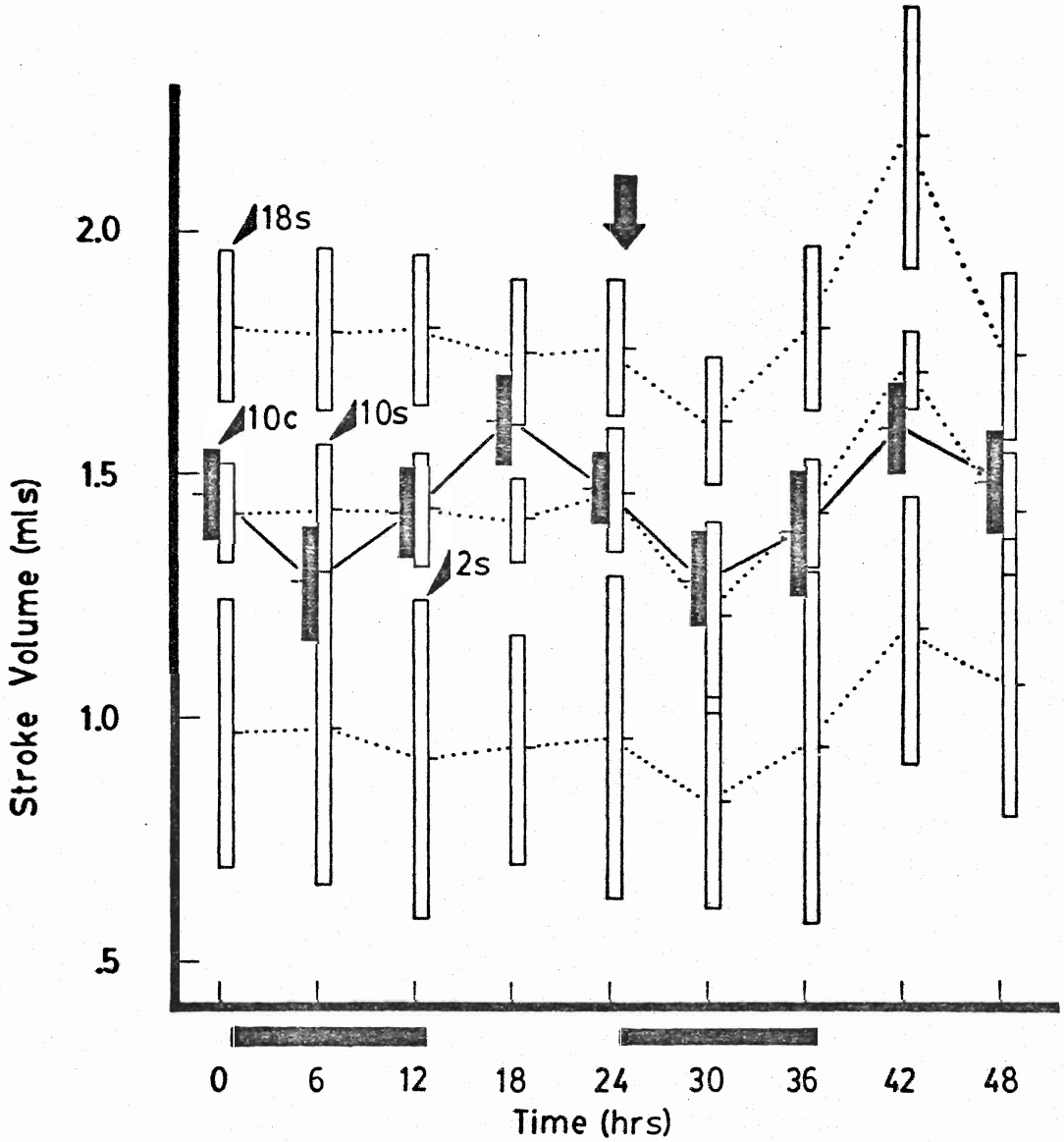
Values recorded for stroke volume (Vsv)(Appendix: Table 8) are summarized in Fig. 29. Vsv increased with temperature by some 1.9 times between 2° and 18°C. Increase in Vsv with temperature, however, decreased with increased acclimation temperature being 1.5 times between 2° and 10°C, and 1.2 times between 10° and 18°C.

Moderate changes in Vsv were observed for statically-acclimated fish

(10° and 18°C) in response to the imposed diurnal temperature cycle. The average Q_{10} values for the cyclic response were 1.59 and 1.50 in 10° and 18°C fish respectively. Fish acclimated to the diurnal temperature cycle also exhibited a moderate cyclic change in Vsv (average Q_{10} - 1.32) which only differed from that of the 10°C at the peak of the cycle (14°C) where the increase in Vsv was slightly greater in the latter group.

With one exception, there were no differences in the response of the fish within any acclimation group to either the upper or lower portion of the diurnal temperature cycle, as indicated by the component Q_{10} values (Table 8). Fish acclimated to 18°C, however, showed a greater response to the upper portion of the cycle (average Q_{10} - 1.68) than the lower portion (average Q_{10} - 1.33). It should be noted that again, large individual variation exists in the data for Vsv, especially for 2°C- fish and 10°C fish, in response to the temperature cycle (see 10°C- fish at 30 hours).

Figure 29. Plot showing the effect of temperature on stroke volume (Vsv, mls). For explanation of symbols used see text for Figure 24.


18s
10c
10s
2s
2.0
1.5
1.0
.5
Stroke Volume (ml s)
0
6
12
18
24
30
36
42
48
Time (hrs)

7. Cardiac-to-ventilatory Rate Ratio.

Values calculated for the cardiac-to-ventilatory rate ratio ($^{CR}/_{VR}$) (Appendix: Table 9) are summarized in Fig. 30. $^{CR}/_{VR}$ did not increase consistently with temperature. The rate ratio increased by some 1.2 times between 2° and 10°C. There were no significant differences between 10°, 18°C, or cycled fish. The mean rate ratio in 18°C- fish, however, was always greater than that for 10°C at constant temperature (Fig. 30).

With one exception, no significant changes were observed in $^{CR}/_{VR}$ in response to cycling temperatures for either statically (10° and 18°C) or cyclically-acclimated trout. It should be noted that small cyclical trends are apparent in each case, but these are rendered nonsignificant due to the large individual variation encountered within all acclimation groups, especially with regards to the absolute magnitude of the ratio. For fish acclimated to 10°C the cyclic response was small (average Q_{10} - 1.27), while for cyclically acclimated fish the trend was less obvious (average Q_{10} - 1.11). Fish acclimated to 18°C showed a slight cyclical response as well, but in this case the rate ratio decreased in response to both the upper and lower portions of the cycle (average Q_{10} - 1.30).

8. Electrocardiogram.

Values recorded from the analysis of the electrocardiographic traces (Appendix: Tables 10 to 13) are summarized at the bottom of Tables 9 through 12 . A typical electrocardiogram is included in Fig. 31.

For descriptive purposes the P-Q, Q-S. and S-T intervals were recorded, as well as the voltage of the QRS complex. The voltage of the waves in the normal ECG will depend on the placement of the electrodes. In this study, the ECG electrodes were placed in the chest cavity in close proximity to the heart. Since the cardiac surfaces are so close to the electrodes, each electrode will record mainly the electrical potential of the heart musculature

Figure 30. Plot showing the effect of temperature on the cardiac-to-ventilatory rate ratio ($^{CR}/_{VR}$). For explanation of symbols used see text for figure 24.

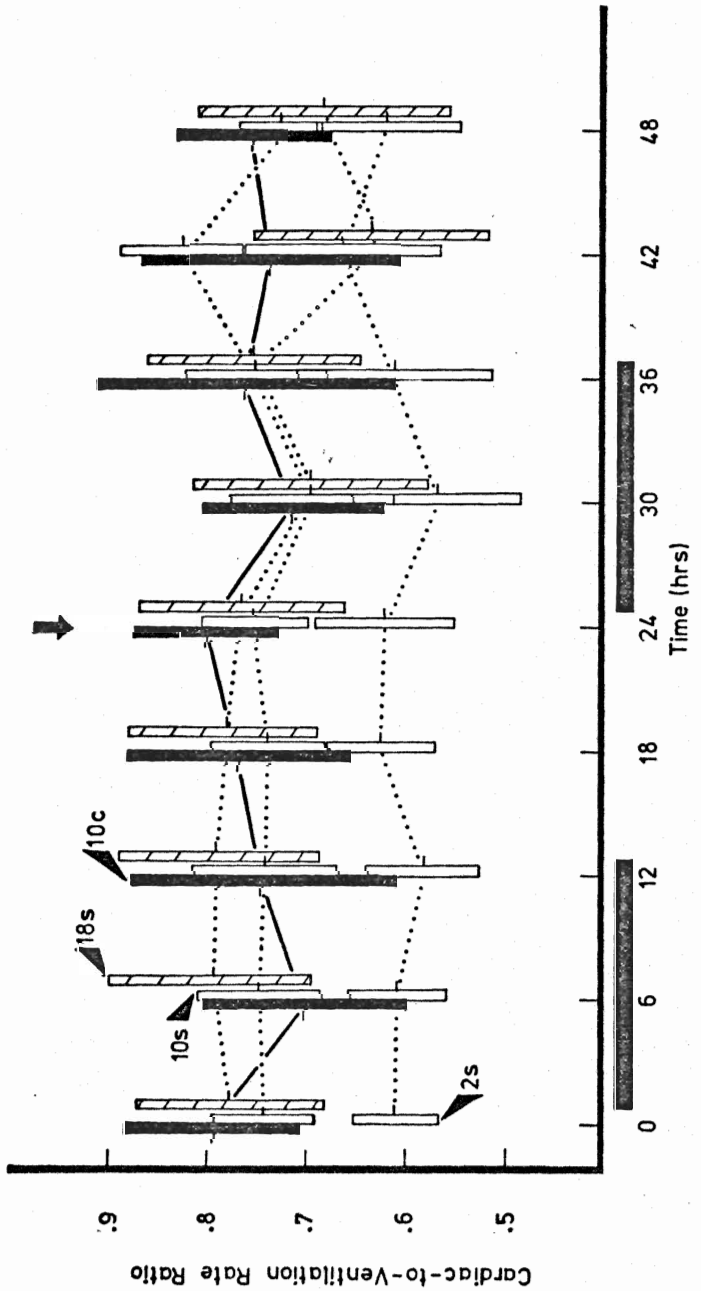

Cardiac-to-Ventilation Rate Ratio
.9
.8
.7
.6
.5
0
6
12
18
24
30
36
42
48
Time (hrs)
2s
10s
18s
10c

Figure 31. Simultaneous recordings of opercular and buccal pressures. The ECG trace is included below. The pressure waveforms from the opercular and buccal respiratory pumps were described in the following manner. For opercular pressure: (1A) -ve amplitude; (1B) +ve amplitude; (1C) area beneath the curve corresponding to -ve pressure; (1D) area beneath the curve corresponding to +ve pressure. For buccal pressure: (2A) +ve amplitude; (2B) -ve amplitude; (2C) area beneath the curve corresponding to +ve pressure; (2D) area beneath the curve corresponding to -ve pressure. 1A and 1C are termed minimum opercular pressure and area respectively. 1B and 1D are termed maximum opercular pressure and area respectively. 2A and 2C are termed maximum buccal pressure and area. 2B and 2D are termed minimum buccal pressure and area respectively.

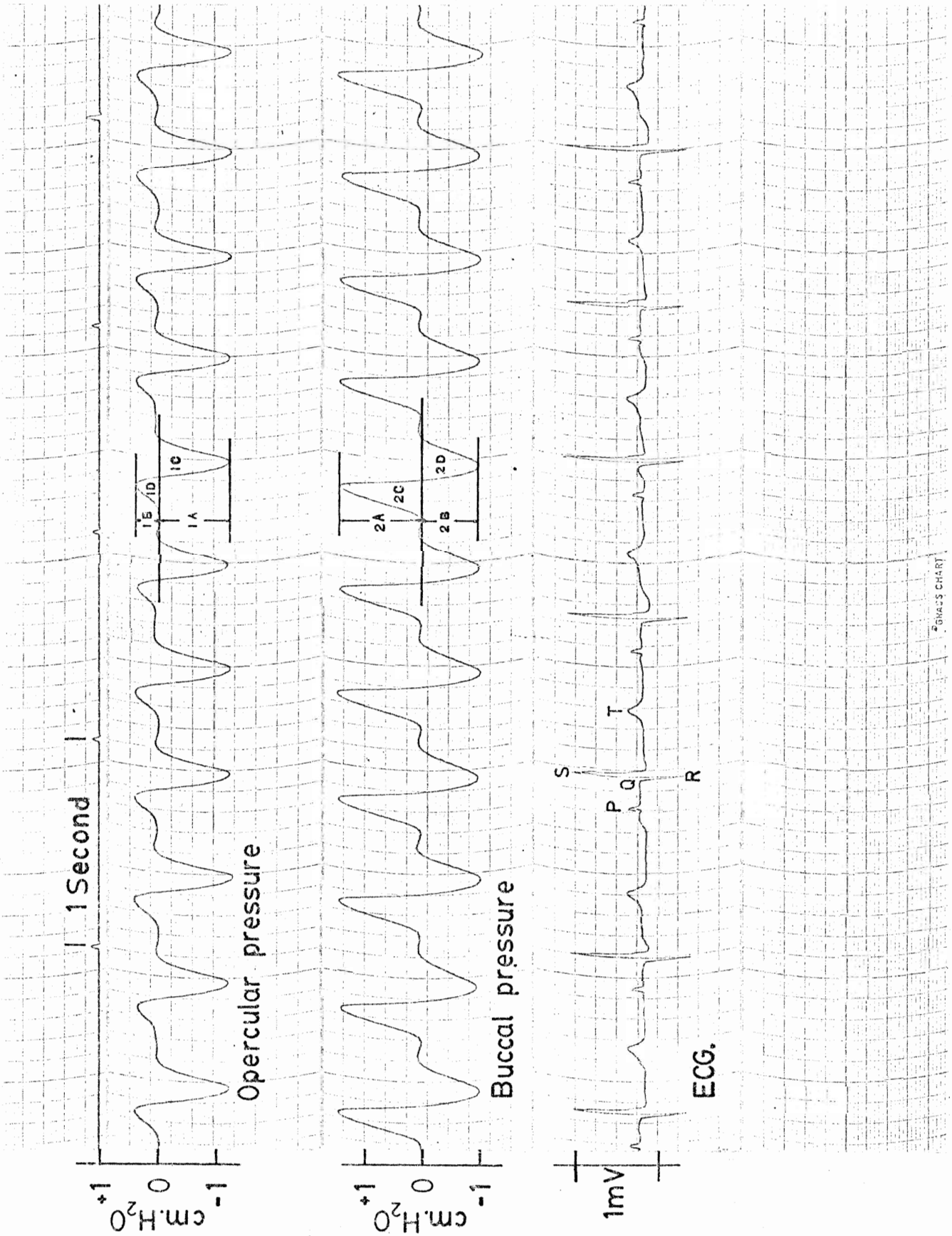

1 Second
Opercular pressure
cm.H2O
+1
0
-1
1A
1B /1D
1C
Buccal pressure
2A
2B
2C
2D
ECG.
1mV
P
Q
R
S
T
GRASS CHART

Table 9. Cardiovascular and respiratory parameters recorded from 10 rainbow trout (371.3 ±7.16 gms) statically acclimated to 2°C. Values are means ± one standard error. Pressures are in mm Hg. Areas are in arbitrary units.

	Time (hours)								
Parameters	0	6	12	18	24	30	36	42	48
Max. buccal pressure	0.49 (.064)	0.62 (.072)	0.68 (.074)	0.62 (.065)	0.69 (.077)	0.64 (.066)	0.64 (.077)	1.02 (.095)	0.69 (.082)
Max. buccal area	0.69 (.085)	0.64 (.070)	0.71 (.075)	0.69 (.086)	0.69 (.062)	0.69 (.062)	0.66 (.064)	1.10 (.077)	0.81 (.058)
Min. buccal pressure	0.23 (.042)	0.29 (.044)	0.32 (.044)	0.33 (.046)	0.30 (.044)	0.31 (.041)	0.30 (.046)	0.49 (.057)	0.30 (.039)
Min. buccal area	0.34 (.083)	0.41 (.078)	0.46 (.069)	0.42 (.050)	0.37 (.053)	0.42 (.053)	0.42 (.075)	0.71 (.094)	0.47 (.077)
Max. operc. pressure	0.37 (.056)	0.40 (.056)	0.42 (.056)	0.40 (.052)	0.45 (.051)	0.45 (.066)	0.50 (.071)	0.65 (.080)	0.47 (.079)
Max. operc. area	0.36 (.058)	0.44 (.047)	0.41 (.050)	0.40 (.050)	0.43 (.043)	0.45 (.054)	0.46 (.056)	0.70 (.092)	0.51 (.076)
Min. operc. pressure	0.37 (.042)	0.42 (.042)	0.47 (.045)	0.50 (.057)	0.56 (.050)	0.53 (.056)	0.52 (.053)	0.72 (.100	0.39 (.056)
Min. operc. area	0.48 (.065)	0.53 (.074)	0.59 (.075)	0.61 (.053)	0.57 (.050)	0.57 (.052)	0.55 (.049)	0.86 (.110)	0.50 (.066)
P-Q interval (sec.)	0.32 (.021)	0.32 (.015)	0.31 (.017)	0.31 (.015)	0.31 (.016)	0.30 (.019)	0.31 (.020)	0.24 (.018)	0.31 (.016)
Q-S interval (sec.)	0.11 (.016)	0.11 (.016)	0.11 (.013)	0.10 (.010)	0.11 (.015)	0.12 (.018)	0.11 (.016)	0.13 (.033)	0.12 (.016)
S-T interval (sec.)	0.65 (.023)	0.65 (.023)	0.66 (.034)	0.62 (.030)	0.65 (.026)	0.65 (.018)	0.65 (.026)	0.47 (.031)	0.66 (.039)
Temp. °C	2.1 (.031)	2.0 (.031)	2.0 (.018)	2.1 (.037)	2.0 (.029)	2.1 (.030)	2.0 (.018)	6.0 (.030)	2.0 (.023)

Table 10. Cardiovascular and respiratory parameters recorded from 10 rainbow trout (407.5 ±11.94 gms) statically acclimated to 10°C. Values are means ± one standard error. Pressures are in mm Hg. Areas are in arbitrary units.

	Time (hours)								
Parameters	0	6	12	18	24	30	36	42	48
Max. buccal pressure	1.23 (.14)	1.29 (.14)	1.27 (.13)	1.34 (.11)	1.27 (.17)	0.53 (.079)	1.27 (.21)	1.90 (.21)	1.24 (.23)
Max. buccal area	1.54 (.13)	1.56 (.12)	1.68 (.14)	1.62 (.13)	1.70 (.21)	0.74 (.11)	1.53 (.22)	2.80 (.30)	1.70 (.32)
Min. buccal pressure	0.31 (.047)	0.31 (.048)	0.35 (.059)	0.32 (.046)	0.33 (.044)	0.12 (.018)	0.33 (.033)	0.60 (.045)	0.33 (.055)
Min. buccal area	0.35 (.058)	0.33 (.060)	0.40 (.075)	0.41 (.063)	0.39 (.062)	0.13 (.023)	0.40 (.051)	0.75 (.073)	0.55 (.110)
Max. operc. pressure	0.38 (.074)	0.35 (.047)	0.37 (.042)	0.38 (.063)	0.31 (.052)	0.10 (.028)	0.24 (.044)	0.60 (.150)	0.24 (.045)
Max. operc. area	0.44 (.067)	0.42 (.067)	0.42 (.060)	0.43 (.064)	0.40 (.079)	0.16 (.045)	0.34 (.076)	0.72 (.180)	0.30 (.054)
Min. operc. pressure	0.58 (.059)	0.59 (.048)	0.62 (.069)	0.57 (.076)	0.48 (.063	0.23 (.053)	0.44 (.077)	1.12 (.280)	0.40 (.073)
Min. operc. area	0.93 (.082)	0.94 (.073)	0.94 (.070)	0.96 (.096)	0.85 (.120)	0.35 (.064)	0.78 (.100)	1.39 (.240)	0.62 (.130)
P-Q interval (sec.)	0.19 (.013)	0.20 (.013)	0.20 (.013)	0.21 (.012)	0.21 (.011)	0.25 (.024)	0.21 (.014)	0.17 (.011)	0.21 (.016)
Q-S interval (sec.)	.07 (.004)	.07 (.005)	.07 (.003)	.06 (.004)	.06 (.002)	.07 (.003)	.06 (.003)	.06 (.002)	.07 (.002)
S-T interval (sec.)	0.40 (.015)	0.41 (.018)	0.42 (.020)	0.45 (.026)	0.43 (.019)	0.55 (.034)	0.45 (.027)	0.33 (.065)	0.46 (.083)
Temp. °C	10.0 (.039)	10.0 (.025)	10.0 (.028)	10.0 (.035)	10.0 (.033)	6.0 (.028)	10.0 (.025)	14.0 (.033)	10.0 (.033)

Table 11. Cardiovascular and respiratory parameters recorded from 10 rainbow trout (414.5 ±13.66 gms) statically acclimated to 18°C. Values are means ± one standard error. Pressures are in mm Hg. Areas are in arbitrary units.

	Time (hours)								
Parameters	0	6	12	18	24	30	36	42	48
Max.buccal pressure	2.25 (.28)	2.01 (.20)	2.24 (.22)	2.33 (.22)	2.43 (.19)	1.73 (.21)	2.24 (.28)	3.12 (.29)	2.12 (.28)
Max.buccal area	3.45 (.54)	3.58 (.66)	3.43 (.37)	3.40 (.42)	3.71 (.51)	2.46 (.35)	3.50 (.59)	4.57 (.58)	3.38 (.46)
Min.buccal pressure	1.63 (.32)	1.35 (.16)	1.71 (.30)	1.59 (.24)	1.80 (.19)	0.92 (.20)	1.71 (.23)	2.54 (.28)	1.61 (.27)
Min.buccal area	1.68 (.29)	1.50 (.24)	1.78 (.34)	1.64 (.24)	1.84 (.20)	0.90 (.15)	1.85 (.26)	2.74 (.46)	1.91 (.37)
Max.operc. pressure	0.96 (.30)	0.81 (.15)	0.83 (.12)	0.97 (.22)	0.90 (.13)	0.88 (.18)	1.13 (.21)	1.26 (.30)	0.92 (.15)
Max.operc. area	1.06 (.22)	1.01 (.17)	1.10 (.16)	0.99 (.12)	1.32 (.22)	1.31 (.34)	1.62 (.38)	2.02 (.57)	1.32 (.29)
Min.operc. pressure	2.58 (.44)	2.39 (.49)	2.66 (.54)	2.33 (.48)	2.70 (.57)	1.64 (.40)	3.03 (.44)	3.37 (.95)	2.73 (.71)
Min.operc. area	2.76 (.42)	2.60 (.43)	2.77 (.55)	2.83 (.49)	3.05 (.53)	1.65 (.40)	3.61 (.63)	4.89 (1.45)	3.01 (.84)
P-Q interval (sec.)	0.11 (.008)	0.12 (.006)	0.11 (.005)	0.12 (.005)	0.11 (.005)	0.13 (.009)	0.11 (.005)	0.09 (.006)	0.11 (.006)
Q-S interval (sec.)	.06 (.003)	.06 (.002)	.06 (.002)	.06 (.005)	.05 (.002)	.06 (.004)	.05 (.002)	.05 (.003)	.06 (.002)
S-t interval (sec.)	0.25 (.015)	0.27 (.016)	0.26 (.016)	0.26 (.014)	0.27 (.013)	0.35 (.015)	0.26 (.015)	0.23 (.019)	0.26 (.024)
Temp. °C	18.0 (.030)	18.0 (.032)	18.0 (.029)	18.0 (.027)	18.0 (.035)	14.0 (.026)	18.0 (.027)	22.0 (.035)	18.0 (.031)

Table12. Cardiovascular and respiratory parameters recorded from 10 rainbow trout (422.5 ±6.53 gms) acclimated to a diurnal temperature cycle (amplitude 8°C,midpoint 10°C). Values are means ± one standard error. Pressures are in mm Hg. Areas are in arbitrary units.

	Time (hours)								
Parameters	0	6	12	18	24	30	36	42	48
Max.buccal pressure	1.03 (.10)	0.56 (.069)	0.79 (.035)	1.59 (.16)	0.85 (.098)	0.57 (.070)	0.84 (.063)	1.51 (.14)	0.87 (.074)
Max.buccal area	1.61 (.10)	0.56 (.084)	0.79 (.10)	1.59 (.25)	0.85 (.17)	0.57 (.086)	0.84 (.15)	1.51 (.31)	0.87 (.15)
Min.buccal pressure	0.50 (.12)	0.17 (.027)	0.38 (.078)	1.22 (.26)	0.41 (.068)	0.18 (.040)	0.40 (.099)	0.99 (.15)	0.46 (.11)
Min.buccal area	0.89 (.14)	0.43 (.08)	0.81 (.11)	1.62 (.23)	0.68 (.11)	0.38 (.11)	0.75 (.11)	1.77 (.22)	0.77 (.12)
Max.operc. pressure	0.50 (.046)	0.37 (.034)	0.46 (.046)	0.68 (.112)	0.52 (.096)	0.37 (.046)	0.51 (.070)	0.66 (.096)	0.40 (.058)
Max.operc. area	0.96 (.17)	0.81 (.18)	0.80 (.17)	0.98 (.14)	0.82 (.16)	0.69 (.15)	0.93 (.22)	0.71 (.14)	0.60 (.091)
Min.operc. pressure	0.84 (.11)	0.41 (.082)	0.82 (.13)	1.54 (.25)	0.78 (.099)	0.38 (.079)	0.83 (.14)	1.58 (.22)	0.84 (.14)
Min.operc. area	1.64 (.28)	0.68 (.10)	1.04 (.20)	2.44 (.30)	1.08 (.16)	0.55 (.11)	1.21 (.17)	2.34 (.34)	1.13 (.20)
P-Q interval (sec.)	0.18 (.010)	0.24 (.013)	0.20 (.014)	0.13 (.007)	0.19 (.015)	0.27 (.025)	0.24 (.048)	0.14 (.012)	0.20 (.016)
Q-S interval (sec.)	.08 (.008)	.11 (.02)	.09 (.01)	.08 (.01)	.09 (.01)	.10 (.02)	.09 (.008)	.07 (.008)	.09 (.01)
S-T interval (sec.)	0.35 (.037)	0.52 (.033)	0.40 (.025)	0.31 (.020)	0.41 (.019)	0.55 (.040)	0.43 (.030)	0.28 (.024)	0.42 (.030)
Temp. °C	10.0 (.037)	6.0 (.026)	10.0 (.031)	14.0 (.031)	10.0 (.027)	6.0 (.031)	10.0 (.030)	14.0 (.026)	10.0 (.031)

adjacent to it. Therefore, relatively minute abnormalities in the cardiac tissues, and slight differences in electrode placement will frequently cause significant individual variation in ECG's recorded from chest electrodes (Guyton, 1966).

This was indeed the case for ECG's recorded via subdermal electrodes in this study. Values for QRS voltage (Appendix: Table 13) showed considerable variation both within and between acclimation groups, especially for fish acclimated to 2°C. Values of less than 0.1 and greater than 1.0 millivolts were obtained in all acclimation groups. It should be noted that excluding the data for 2°C- fish which was extremely variable, there was a nonsignificant trend for QRS voltage to increase with increased acclimation temperature between 10° and 18°C.

The P-Q interval - the period of time between the onset of contraction in the atrium (P wave) and ventricle (QRS complex) - decreased with temperature by some 2.79 times between 2° and 18°C. The decrease in the P-Q interval increased with increased acclimation temperature, being 1.55 times between 2° and 10°C, and 1.80 times between 10° and 18°C.

Moderate changes in the P-Q interval were observed in trout acclimated to 10° and 18°C in response to the imposed diurnal temperature cycle. The average Q_{10} value for the cyclic response was 1.61 for both 10°C and 18°C fish. Fish acclimated to the diurnal temperature cycle also exhibited a moderate cyclic response in the P-Q interval (average Q_{10} - 2.17) which differed from that of the 10°C trout only at the highest point of the cycle (14°C). The cycled fish exhibited a significantly greater decrease in P-Q with increased temperature during the upper portion of the cycle.

For rainbow trout acclimated to 10° and 18°C there was very little difference in the response to the upper or lower portion of the temperature cycle. Cyclically acclimated fish, however, showed a much greater response

to the upper portion of the cycle than the lower portion, as indicated by the component Q_{10} values. (Table 8).

The Q-T interval - the period of time corresponding to the contraction of the ventricle between depolarization (QRS) and repolarization (T wave) is described by the Q-S and S-T intervals given in Tables 9 through 12. The changes in Q-S observed were generally small and nonsignificant, especially within acclimation groups, although there was a definite trend for Q-S to decrease with increased acclimation temperature (1.8 times between 2°and 18°C). Decrease in Q-S interval decreased with increased acclimation temperature being 1.57 times between 2° and 10°C, and 1.20 times between 10° and 18°C. There were no significant cyclical changes in Q-S in response to the imposed temperature cycle. The changes in the S-T interval, on the other hand, were significant both within and between acclimation groups. The S-T interval decreased with increased temperature by some 2.5 times between 2° and 18°C. The magnitude of the change in S-T remained fairly constant with increased acclimation temperature being 1.55 times between 2° and 10°C; and 1.62 times between 10° and 18°C.

Moderate changes in S-T were observed in fish acclimated to 10°C in response to the imposed diurnal temperature cycle. Trout acclimated to the temperature cycle, also exhibited a moderate cyclic response in S-T which did not differ significantly from that of 10° C- fish. The average Q_{10} values for the cyclic response were 1.98 and 2.18 for the 10°C and cycled fish respectively. Trout acclimated to 18°C, however, did not decrease the S-T interval significantly in response to the upper portion of the temperature cycle (Table 11). 18°C fish did, however, exhibit a moderate increase in S-T in response to the lower portion of the cycle. With the exception of 18°C- fish, there were no differences in the response of the trout to either the upper or lower portion of the cycle in terms of the S-T interval (10°C and cycled- fish).

9. Pressure Waveforms of Respiratory Pumps

Values recorded from the analysis of pressure waveforms (Appendix: Tables 14 to 21) are summarized in Tables 7 through 10. A typical trace of buccal and opercular pressures is included in Fig. 31 along with an explanation of the descriptive analysis used.

For descriptive purposes pressure waveforms were divided into maximum and minimum components depending on whether the pressure generated was +ve or -ve with respect to ambient water pressure (Fig. 31). A minimum pressure (or area), therefore, represents a negative pressure (suction) in the respective respiratory cavities while a maximum pressure represents a positive pressure or propulsive force in the respiratory cavities.

Good agreement was obtained between measurements of amplitude and area from pressure waveforms. The data as a whole, however, showed significant variation, especially for those components which were of very small magnitude and labile in character (e.g., maximum opercular and minimum buccal components). For this reason some overlap was observed in certain components between acclimation groups at constant temperature (eg., 2° C and 10°C).

In general, buccal and opercular pressures increased markedly with acclimation temperature. The maximum buccal component (+ve pressure) increased 4 to 5 times between 2°C and 18°C. The magnitude of the increase in the maximum buccal component remained constant with increased temperature being approximately 2 times between both 2° and 10°C, and 10° and 18°C. The minimum opercular component (-ve pressure), however, remained fairly constant between 2° and 10°C (increased 1.4 times), but increased approximately 5 times between 10° and 18°C. The minimum buccal component (-ve pressure), and maximum opercular component (+ve pressure) were small in magnitude, and did not differ significantly between 2° and 10°C. Both components, however, increased substantially between 10° and 18°C by some 2.45 and 4.92 times respectively.

Marked changes in respiratory pressures were observed for trout acclimated to 10° and 18°C in response to the imposed diurnal temperature cycle. The Q_{10} values obtained for these cyclical changes showed wide variation, especially for smaller pressure components (Table 8). The average Q_{10} values for the buccal and opercular pressure components were approximately 8.0 and 3.0 in 10° and 18°C- fish respectively. It should be noted that individual variation in 18°C fish was greater than in 10°C- fish, and in some cases rendered the changes observed non-significant, especially at higher temperatures.

Fish acclimated to the temperature cycle, also exhibited a marked response in buccal and opercular pressures (average Q_{10} - 5.0). The response of the cycled fish differed from that of 10°C fish in that recorded pressures were generally somewhat greater, with the exception of the maximum buccal component which, in general, did not differ significantly between the two groups. It is felt that this was due to the smaller variation in values obtained for fish acclimated to 10°C.

The magnitude of the pressure response to either the upper or lower portion of the cycle varied with acclimation temperature. Trout acclimated to 10°C showed the greatest response in buccal and opercular pressure (average Q_{10} - 8.0) to the cycle as a whole. In addition, however, the buccal pressure in 10°C fish exhibited a markedly greater response to the lower portion of the cycle (average Q_{10} - 11.1) than the upper portion (average Q_{10} - 3.70). The opercular pressure did not exhibit a differential response (average Q_{10} - 8.0). Trout acclimated to 18°C exhibited a much less pronounced response in pressure (average Q_{10} - 3.0), and there was little difference in the magnitude of observed pressure changes for the upper and lower portions of the temperature cycle. Trout acclimated to the temperature cycle exhibited a cyclic pressure response which was intermediate in magni-

tude (average Q_{10} - 5.0) between that of statically acclimated fish at 10° and 18°C, and was nondifferential with respect to the cycle.

The relative contribution of the opercular and buccal pumps varied between acclimation temperatures, and within acclimation groups between individuals. For fish acclimated to 2°C the contribution of the buccal pump was slightly greater than that of the opercular pump to the total respiratory pressure. For 10°C fish the buccal pump was definitely dominant (2 fold greater than the opercular pump). For 18°C fish, and for fish acclimated to the temperature cycle, however, the two pumps contributed almost equally to the total respiratory pressure. These relationships were found to be generally true throughout the study for both constant and cycling temperature.

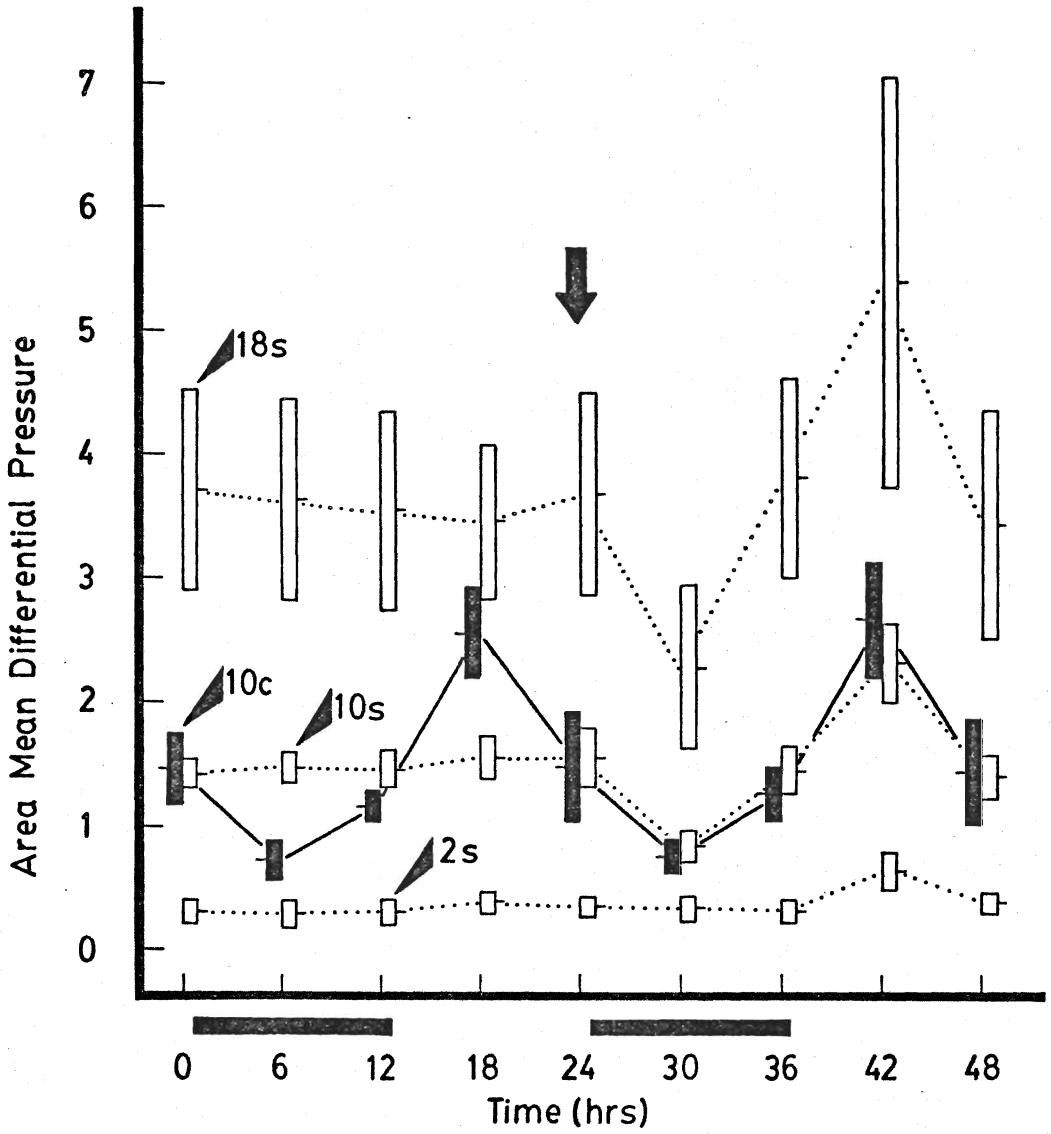
10. Area Mean Differential Pressure.

Values recorded for the area mean differential pressure (MDP) (Appendix: Table 22) are summarized in Fig. 32. MDP increased with temperature by some 10.9 times between 2° and 18°C. Increase in MDP decreased with increased acclimation temperature, being 4.5 times between 2° and 10°C, and 2.4 times between 10° and 18°C.

Marked changes in MDP were observed in statically acclimated trout (10° and 18°C) in response to the imposed temperature cycle. The average Q_{10} values for cyclical changes were 3.93 and 3.10 and 10° and 18°C- trout respectively. Fish acclimated to the temperature cycle also exhibited a marked cyclic response in MDP (average Q_{10} O 4.57), which although slightly greater in magnitude, did not differ significantly from that observed for 10°C- fish.

There did not appear to be any difference in the response of trout within any of the acclimation groups to either the upper or lower portion of the temperature cycle as indicated by the component Q_{10} values (Table 8.)

Figure 32. Plot showing the effect of temperature on the area mean differential pressure (MDP, arbitrary units). The area mean differential pressure is equal to the algebraic sum of the area mean differential components (a + b - c) beneath the differential pressure curve. (a) is the opercular suction pump component, (b) is the buccal force pump component, and (c) the reversal component of the area mean differential pressure (arbitrary units). For explanation of symbols used see text for Figure 24.


Area Mean Differential Pressure
7
6
5
4
3
2
1
0
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

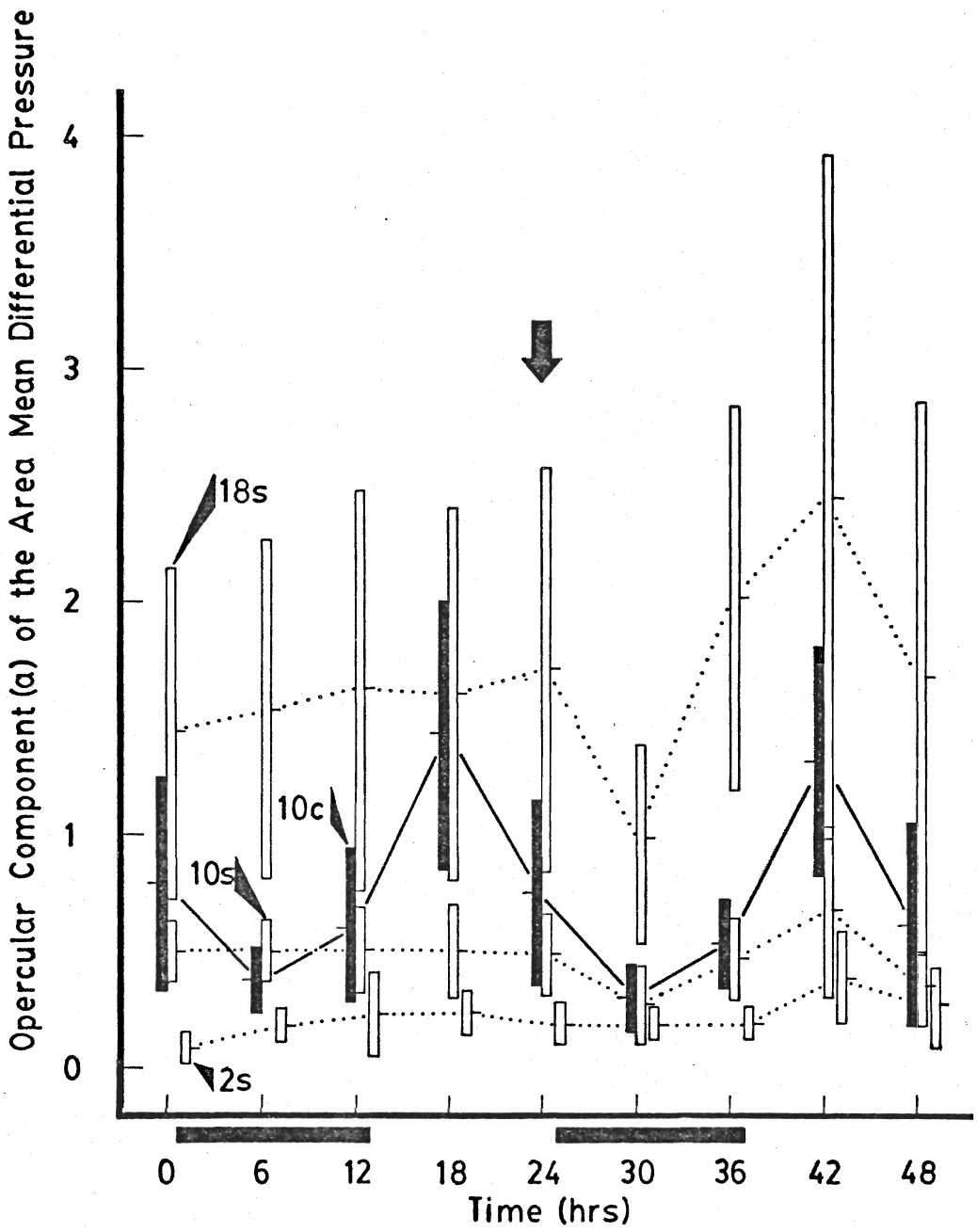
11. Opercular Component (a) of the Area Mean Differential Pressure.

Values recorded for opercular component (a) of the MDP (Appendix: Table 23) are summarized in Fig. 33. The opercular component (a) increased with temperature by some 8.3 times between 2° and 18°C. The increase in (a) increased with acclimation temperature being 2.6 times between 2° and 10°C, and 3.2 times between 10° and 18°C. It should be noted that individual variation within acclimation groups at constant temperature increased with the temperature of acclimation. The variation was particularly large for trout acclimated to 18°C.

Large individual variation was also found within acclimation groups in response to the diurnal temperature cycle. The magnitude of the variation rendered many cyclical changes nonsignificant, especially at high temperatures, both within and sometimes between acclimation groups (Fig. 33).

Marked changes in (a) were observed in statically acclimated fish (10°C and 18°C) in response to the imposed temperature cycle. The average Q_{10} values for these changes were 4.75 and 3.69 for 10° and 18°C- fish respectively. Due to large variation in the values of (a) obtained in 18°C- trout, however, the cyclic increase in (a) at the peak of the cycle was not significantly greater than the values of (a) recorded at 18°C. It would appear that a few of the 18°C trout were unable to either increase or maintain the opercular component (a) in the face of the cyclic temperature increase. Large variation in values for 18°C fish at 22°C and 18°C (42 and 48 hrs) indicated that some fish had virtually lost the opercular component of mean differential pressure during the upper portion of the cycle. Fish acclimated to the temperature cycle also exhibited a marked cyclic response in (a) (average Q_{10} - 6.78), which differed from that of the 10°C fish in that (1) the increase in (a) was greater in response to the upper portion of the cycle (Q_{10} - 9.19), and

Figure 33. Plot showing the effect of temperature on the opercular pump component (a) of the area mean differential pressure (a, arbitrary units). For explanation of the symbols used see text for Figure 24.


Opercular Component (a) of the Area Mean Differential Pressure
4
3
2
1
0
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

(2) individual variation was greater. The nature of the variation indicated that some cycled fish generally exhibited higher values for (a), especially at high temperatures (Fig. 33).

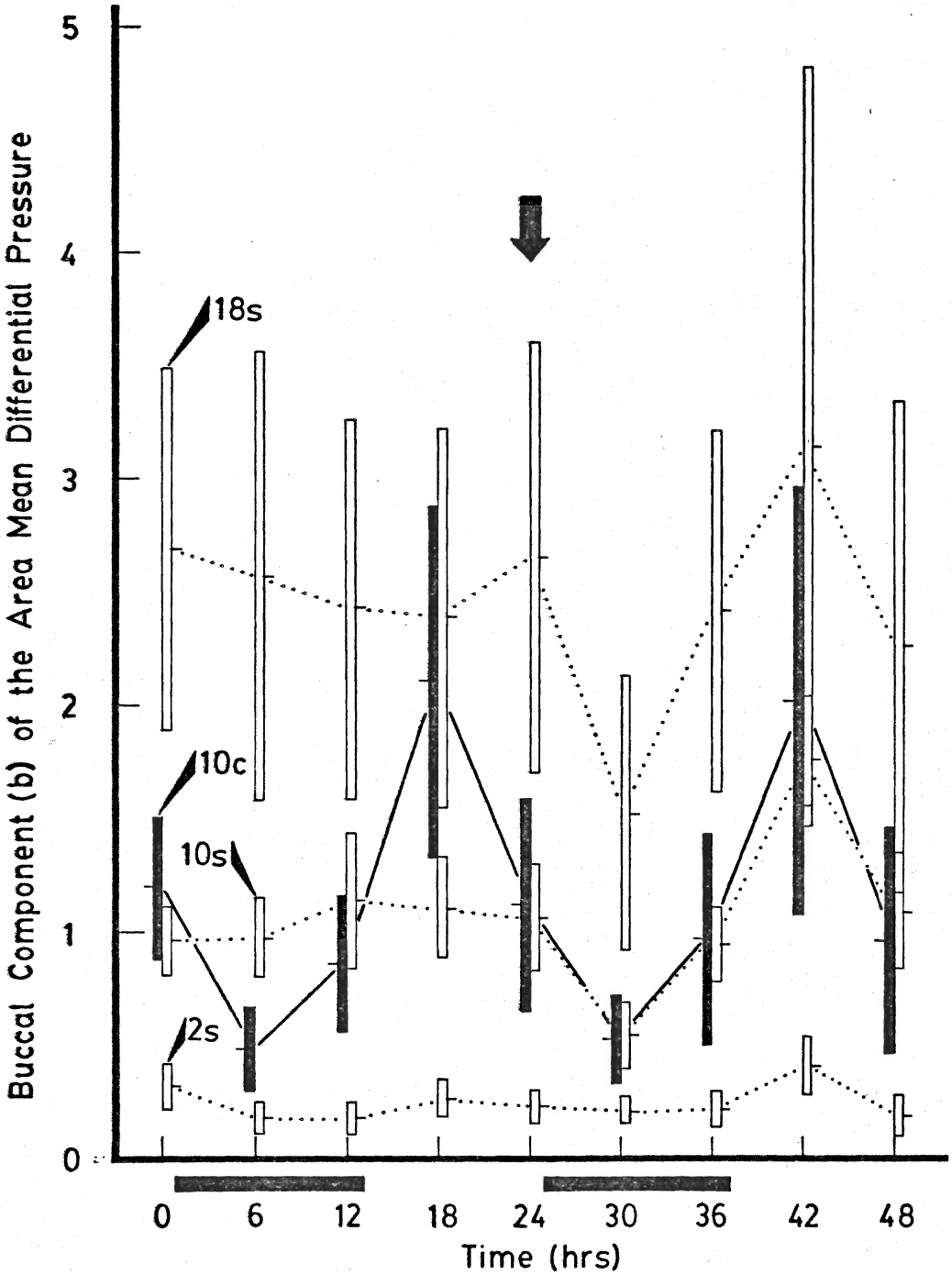
There was little difference in the response of 10°C or cycled fish to either the upper or lower portion of the temperature cycle, with the possible exception of a slightly greater response to the upper portion, especially for cycled fish. (Table 8). Fish acclimated to 18°C, however, exhibited a greater response to the lower portion of the cycle (average Q_{10} - 5.28).

12. Buccal Component (b) of the Area Mean Differential Pressure.

Values recorded for the buccal component (b) of the MDP (Appendix: Table 24) are summarized in Fig. 34. The buccal component (b) increased with temperature by some 10.6 times between 2° and 18°C. Increase in (b) however, decreased with increased acclimation temperature, being 4.4 times between 2° and 10°C, and 2.4 times between 10° and 18°C. Individual variation within acclimation groups was large, and increased with acclimation temperature as well as with cyclic increases in temperature.

Marked changes in (b) were observed in statically acclimated fish (10° and 18°C) in response to the imposed temperature cycle. The average Q_{10} values for these changes were 4.27 and 2.85 for 10° and 18°C fish respectively. As with opercular component (a), wide variation was found in (b) for 18°C fish, especially at the highest temperature (22°C). It appeared, as with (a), that a few fish were unable to increase or maintain the buccal component (b) in the face of the cyclic temperature increase. Fish acclimated to the temperature cycle also exhibited a marked cyclic response in (b) (average Q_{10} - 6.53) which differed from that of the 10°C-fish in that the individual variation was much greater, especially as temperature increased. The nature of the variation did not allude to any

Figure 34. Plot showing the effect of temperature on the buccal component (b) of the area mean differential pressure (b, arbitrary units). For explanation of the symbols used see text for Figure 24.


Buccal Component (b) of the Area Mean Differential Pressure
5
4
3
2
1
0
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

particular trend in cycled fish as compared to the statically acclimated fish at 10°C.

There was little difference in the response of 10°C or cycled fish to either the upper or lower portion of the temperature cycle (Table 8). Fish acclimated to 18°C, however, exhibited a greater response to the lower portion of the cycle (average Q_{10} - 3.61). The general conclusions which can be drawn with respect to the relative contribution of opercular component (a) and buccal component (b) to mean differential pressure are as follows. (1) The relative difference between (a) and (b) increases with acclimation temperature. At 2°C both components contribute equally to MDP. At 10°C, the contribution by (b) is 2.1 times greater, and at 18°C, 1.61 times greater. (2) With two exceptions the relative change in (a) and (b) in response to cycling temperatures is equal. The relative change in (a) in response to the upper portion of the imposed cycle in 10°C- fish, and to the lower portion of the cycle in cycled fish was less than that of (b), particularly in the former case.

Values for the reversal component (c) (Appendix: Table 25) were generally very small. The estimates of (c) for 2° and 10°C trout were not significantly different, being generally less than 0.1 respiratory units. The reversal component for fish acclimated to 18°C, however, was significantly greater being equal to 0.52 respiratory units at constant temperature. Some nonsignificant cyclical trends in (c) were observed in rainbow trout acclimated to static temperatures (10° and 18°C) in response to cycling temperatures. Fish acclimated to cycling temperatures, however, exhibited a significant cyclical change in (c). The reversal component was generally greater in cyclically acclimated trout compared to statically acclimated fish at any coincident temperature.

13. Relative Minute Volume.

Values recorded for relative minute volume (RMV) (Appendix: Table 26) are summarized in Fig. 35. RMV increased with temperature by some 10.9 times between 2° and 18°C. The increase in RMV decreased with increased acclimation temperature, being 4.5 times between 2° and 10°C, and 2.4 times between 10° and 18°C.

Marked changes in RMV were observed in statically acclimated fish (10° and 18°C) in response to the imposed temperature cycle. The average Q_{10} values for these changes were 3.95 and 3.07 for 10° and 18°C fish respectively. Fish acclimated to the temperature cycle, also exhibited a marked cyclical change in RMV which did not differ significantly from that of 10°C- fish.

There did not appear to be any great difference in the response of rainbow trout within any acclimation group to either the upper or lower portion of the temperature cycle as indicated by the component $Q_{10's}$ (Table 8). The Q_{10} values for the lower portion, however, were generally slightly greater.

14. Gill Resistance

Values recorded for gill resistance (Appendix: Table 27) are summarized in Fig. 36. Gill resistance increased with temperature by some 2.6 times between 2° and 18°C. Increase in gill resistance, however, decreased with increased acclimation temperature being 2.1 times between 2° and 10°C, and 1.3 times between 10° and 18°C.

Moderate changes in gill resistance were observed in fish acclimated to 10°C in response to the imposed temperature cycle (average Q_{10} - 1.78). Fish acclimated to cycling temperatures also exhibited a moderate cyclic response in GR (average Q_{10} - 2.20), which differed from that of 10°C- trout in that individual variation was greater, especially at high temperatures. In fish acclimated to 18°C individual variation was larger, and consequently cyclical

Figure 35. Plot showing the effect of temperature on the relative minute volume (RMV, (a + b - c) x VR, arbitrary units), where (a + b - c) equals the algebraic sum of the area mean differential components beneath the differential pressure curve. For explanation of symbols used see text for Figure 24.

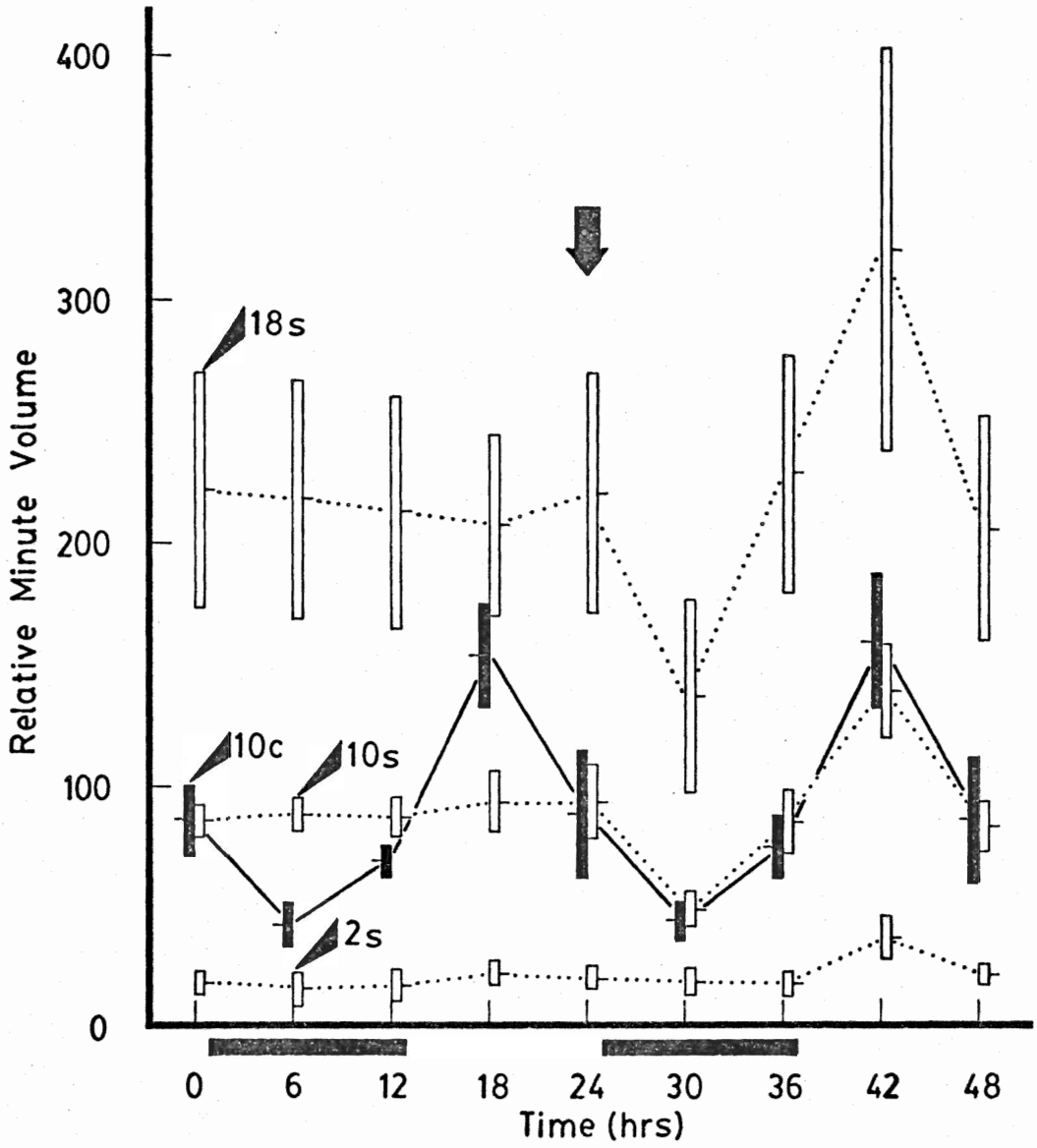
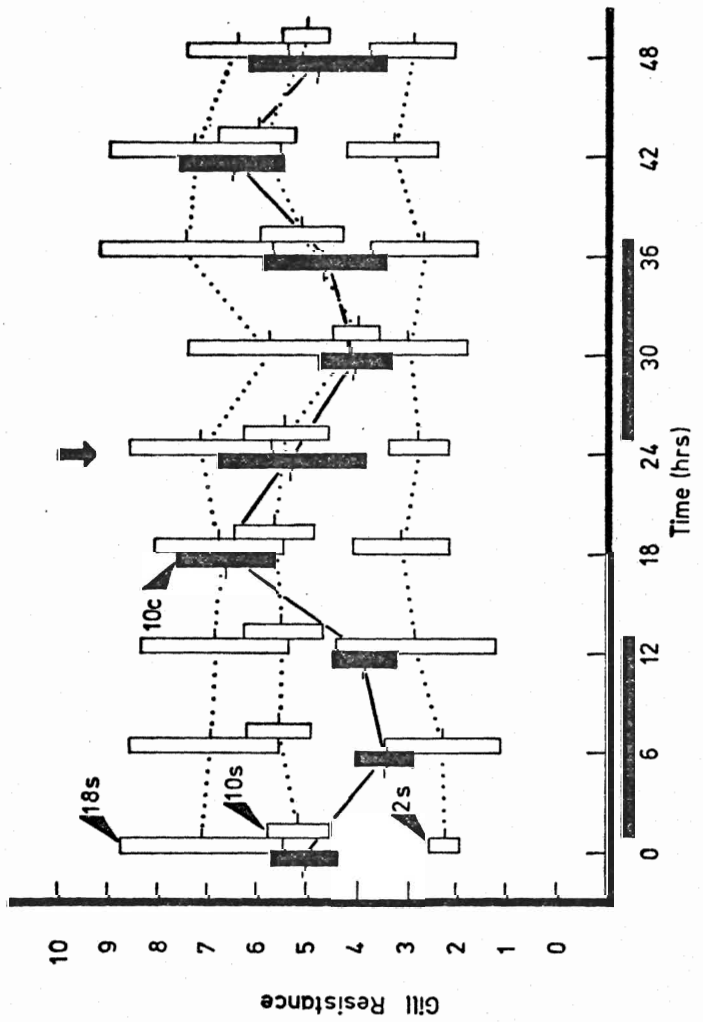

400
300
200
100
0
Relative Minute Volume
18s
10c
10s
2s
0
6
12
18
24
30
36
42
48
Time (hrs)

Figure 36. Plot showing the effect of temperature on gill resistance (GR, arbitrary units) as calculated by dividing the area mean differential pressure (MDP, arbitrary units) by the minute volume ($\dot{V}_G$, l Kg^{-1} min^{-1}). For explanation of symbols used see text for Figure 24.


Gill Resistance
10
9
8
7
6
5
4
3
2
1
0
18s
10s
2s
10c
0
6
12
18
24
30
36
42
48
Time (hrs)

changes in GR were not significantly different from values recorded at constant temperature. It was apparent, however, that 18°C trout decreased GR in response to the lower portion of the cycle, but were unable to increase gill resistance at higher temperatures (18° - 22°C).

The observed cyclical changes were indeed small and irregular in most cases for all acclimation groups (Fig. 36).

16. Phase Relationships between Heart Beat and Ventilatory Pressures.

Although relevent data has not been included, the heart did not appear to beat during any particular phase of the ventilatory cycle (as indicated by the pressure waveforms of the respiratory cavities). No phase relationships were obvious for thermally acclimated trout at constant temperature or during the imposed diurnal temperature cycle within any acclimation group.

DISCUSSION

DISCUSSION

1. Oxygen consumption at constant temperature

Average values for the major cardiovascular-respiratory parameters recorded from thermally-acclimated rainbow trout at 2°, 10°, and 18°C are summarized in Table 13 and Fig. 37. The values for oxygen consumption obtained in this study correspond well with those previously reported for rainbow trout (Table 5). The values of oxygen consumption recorded here should be considered as routine, since quantitative measurements of activity were not made. It should be noted, however, that stringent precautions were taken to reduce any undue disturbance of the fish from extraneous sources. The trout of all groups remained quiescent, resting on the bottom of the respirometer chambers. With the exception of infrequent struggling on the part of a few individuals, no overt movements were observed despite the fact that individuals were only loosely restrained. The fish appeared, for all intents and purposes, to be resting normally.

Oxidative metabolism generally increases in an approximately exponential fashion with acclimation temperature in teleosts (Fig. 11). The apparent nonlinearity of the increase in $\dot{V}_{O_2}$ with acclimation temperature (on a semi-logarithmic plot) in this study agrees with this relationship. The effect of temperature on $\dot{V}_{O_2}$ decreased with increased acclimation temperature, indicating that some form of compensation had taken place to reduce the thermal sensitivity of the trout.

The thermal sensitivities of the various other physiological rate functions (e.g., ventilation rate) can also be described from the semilogarithmic plot in Fig. 37. Although statistical evidence is lacking with the exception of ventilation rate (VR) all cardiovascular-ventilatory rate functions appeared to exhibit decreased thermal sensitivity with increased acclimation temperature. Ventilation rate, on the other hand, exhibited a

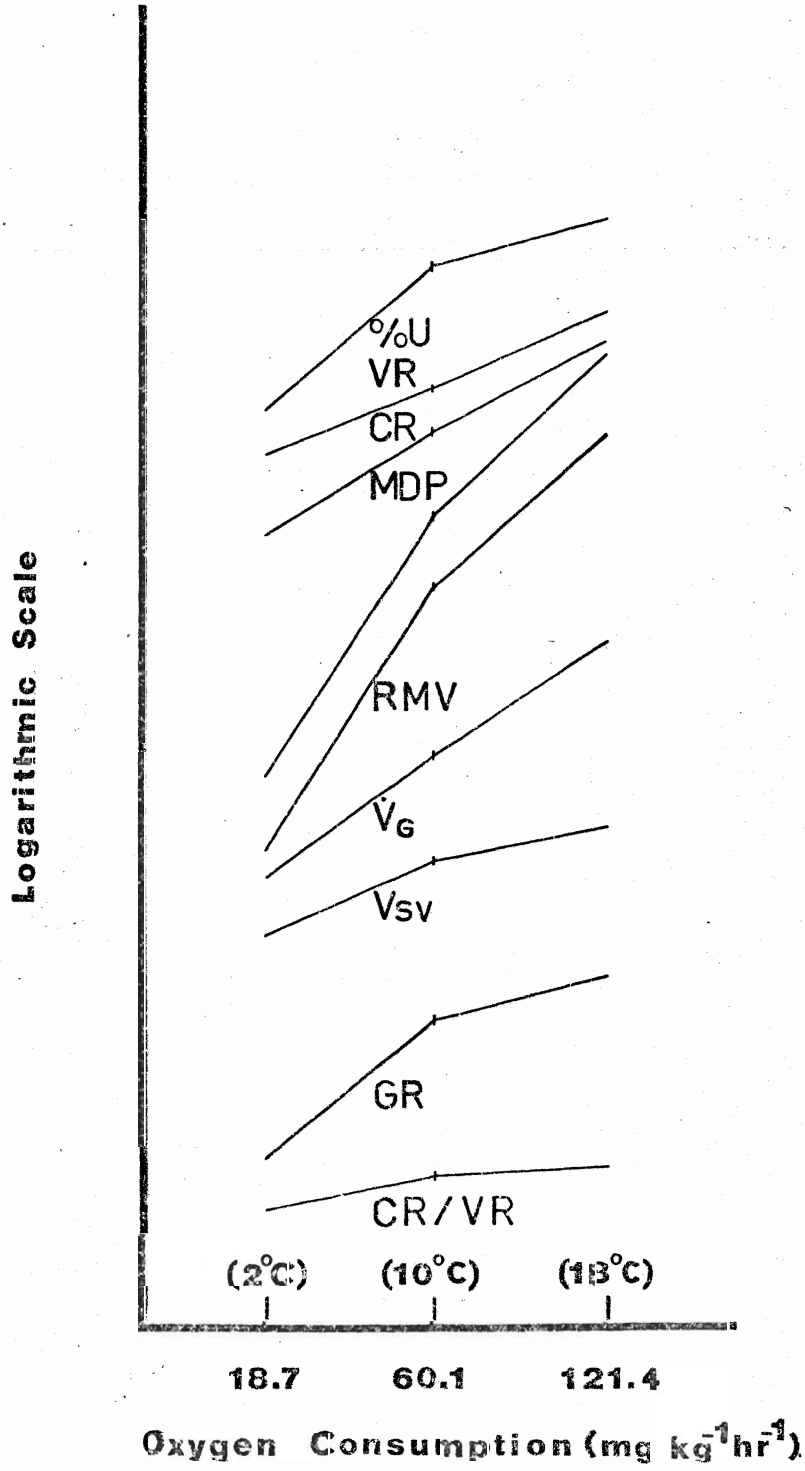
Table 13. Summary of recorded values for cardiovascular and respiratory parameters in thermally- acclimated rainbow trout at static temperatures (i.e., 2°, 10°, and 18°C)

Parameter *	Acclimation Temperature 2°C	10°C	18°C
$\dot{V}_{O_2}$, mg $kg^{-1}hr^{-1}$	18.7	60.1	121.4
% U ,	18.9	42.0	55.9
RMV , arbitrary units	19.7	89.5	215.6
$\dot{V}_G$, l $kg^{-1}min^{-1}$	0.135	0.268	0.514
Vsv , mls	0.95	1.43	1.78
GR	2.63	5.45	6.94
MDP , arbitrary units	0.33	1.49	3.59
Opercular comp., " "	0.19	0.50	1.58
Buccal comp., " "	0.24	1.05	2.55
VR , cycles min^{-1}	54.1	78.5	120.0
CR , No min^{-1}	32.7	57.2	92.5
CR/VR	0.604	0.729	0.771
P-Q interval , sec	0.31	0.20	0.11
Q-S interval , sec	0.11	0.07	0.06
S-T interval , sec	0.65	0.42	0.26
$\dot{V}_G$/CR **	1.53	1.91	2.30

* For an explaination of the symbols used see Materials and Methods section 6.

** $\dot{V}_G$ used in the calculation was in mls min^{-1} (see Appendix: table 4).

Figure 37. Semi-logarithmic plot showing the degree of change in selected ventilatory-cardiovascular rate functions with increased oxygen consumption associated with acclimation to higher temperatures (2°, 10°, and 18°C). The temperature of acclimation is included in brackets. The various parameters are arranged arbitrarily on the logarithmic axis. For an explanation of the symbols used see Materials and Methods, section 6.


Logarithmic Scale
%U
VR
CR
MDP
RMV
$\dot{V}_G$
$\dot{V}_{SV}$
GR
CR/VR
(2°C)
(10°C)
(18°C)
18.7
60.1
121.4
Oxygen Consumption (mg kg^{-1}hr^{-1})

slight increase in thermal sensitivity with temperature.

2. Cardiovascular-Respiratory Parameters at Constant Temperature.

The various cardiovascular-ventilatory parameters recorded are in good agreement with literature values (Table 5) for resting rainbow trout. It is apparent, however, that considerable variation exists in reported estimates of the various parameters. Although significant variation is commonly found in studies of this nature, it is likely that differences in experimental conditions account for many disparities. In each case differences in fish weight, level of activity, holding conditions, oxygen tensions, carbon dioxide tensions, and particularly temperature make direct comparisons between studies difficult unless these factors are taken into account.

Estimates of ventilatory flow ($\dot{V}_G$), ventilatory stroke volume (Vsv), and utilization (%U) involved sampling from midcleithral cannulae and use of the Fick principle. Indirect methods, such as this, assume that the water sampled from the postbranchial region is representative of the mixed expiratory flow leaving the gills. Davis and Watters (1970) have questioned this assumption, and regard it as invalid in some cases. They conclude that use of cannulation techniques to determine mean expired oxygen tensions can lead to serious errors, especially with regards to utilization. For this reason, direct methods of separating inspired from expired water were developed (Davis & Cameron, 1970). The estimates of $\dot{V}_G$, Vsv, and %U obtained here, therefore, are primarily of value in relative terms and should be viewed with some caution.

In contrast to the findings of Davis and Watters (1970) there appeared to be a reasonably high level of precision in any individual fish in estimates of expired oxygen levels at constant temperature. The accuracy of such measurements, however, should be checked by some independent means as suggested by Davis and Watters (1970). It should be noted, however, that the values for

$\dot{V}_G$, Vsv, and %U obtained in this study for rainbow trout at 10°C correspond fairly well with those reported by Davis and Cameron (1970) for rainbow trout at 9°C fitted with oral membranes to directly measure ventilatory parameters.

The results summarized in Fig. 37 indicate some of the responses used by thermally-acclimated rainbow trout to meet the increased $\dot{V}_{O_2}$ and decreased oxygen availability associated with exposure to higher temperatures. Oxygen solubility (α) decreases approximately 40% with an increase in acclimation temperature between 2° and 18°C. Observed increase in oxygen uptake appears to have been achieved by means of rate changes in the several ventilatory and cardiovascular activities involved in the convection and diffusion of oxygen to the sites of oxygen demand in the tissues.

The rainbow trout, for example, increased ventilatory flow ($\dot{V}_G$) nearly exponentially with acclimation temperature. This change was achieved by way of increases in both ventilatory rate (VR) and stroke volume (Vsv), with rate changes in the former being greater in response to the increase in $\dot{V}_{O_2}$ between 10° and 18°C.

The time course of the pressure changes in the respiratory cavities was recorded in an attempt to elucidate the response of the buccal and opercular pumps to acute as well as chronic changes in temperature. Hughes and Shelton (1958) have proposed that the respiratory system in teleosts consists of three chambers, the opercular cavities separated from the buccal cavity by the gill sieve which offers a functional resistance to water flow. They concluded that the breathing pattern of rainbow trout represents a general, unspecialized ventilatory mechanism. The opening and closing of the mouth is associated with the negative and positive aspects of the buccal pressure waveform, with the latter being more prominent. Adduction and abduction of the operculum are associated with the positive and negative opercular pressures, with the latter being more prominent in this case. The opening and closing of the mouth

precede respectively abduction and adduction of the operculum by approximately one-fifth of a ventilatory cycle (Hughes & Shelton, 1958). The buccal and opercular pressure waveforms observed in this study correspond closely to those described above (Fig. 31; Tables 7 to 10).

The differential pressure curve obtained by superimposing and subtracting the buccal and opercular pressures (Fig. 22 and 23), reveals two maxima in each respiratory cycle. In accordance with the description of Hughes and Shelton (1958) the respiratory cycle for rainbow trout in this study is made up of four phases. These are : (1) opercular suction pump predominant (the minimum oeprcular pressure is more negative than the minimum buccal pressure); (2) transition with a reduction in differential pressure (generally a reversal); (3) buccal force pump predominant (the maximum buccal pressure is more positive than the maximum opercular pressure); and (4) transition with a reduction in differential pressure (generally to zero or slightly negative). Differential pressure reversals observed for trout in this study were generally small (<0.1 units at 2° and 10°C; and ≃ 0.5 units at 18°C, arbitrary units), and occurred mainly during phase 2. With the exception of a brief period in this phase when pressure reverses, therefore, the pressure in the buccal cavity exceeds that in the opercular cavity throughout the respiratory cycle. As was the case in earlier studies (Hughes & Shelton, 1958) it is concluded that a unidirectional flow of water is maintained over the gills during almost the entire respiratory cycle. Hughes (1961) added that the observed pressure reversal is so brief, and of such small magnitude, that an actual reversal of water flow is unlikely due to inertial effects.

The relative contribution of the buccal and opercular pressures to ventilatory flow may be determined by either the area underneath the appropriate components of the differential pressure curve, or the amplitude of the buccal and opercular pressure components (Hughes & Roberts, 1970; Hughes & Saunders,

1970; Heath and Hughes, 1973). The former method was adopted for this study. The magnitude of the differential pressures observed in this study (Table 11), although in arbitrary units, appear to correspond quite well with literature values. (~1 to 2 cm H_2O). With respect to the relative contribution of the buccal and opercular pumps, the former was generally dominant, especially at 10°C. Hughes and Shelton, (1958), however, found the pumps to be fairly well balanced in rainbow trout. It should be noted that in both studies the relative contribution of the pumps varied between individuals, and in individual fish with time.

The presence of a semi-constant (in terms of direction) pressure differential indicates that significant resistance to flow exists across the gills. It has been proposed (Hughes & Shelton, 1958; Hughes, 1961; Shelton, 1970; Ballintyn, 1972; Hughes & Morgan, 1973) that teleosts actively expand and contract the gill sieve in response to pressure and volume changes in the respiratory cavities. It is believed that gill resistance varies during single respiratory cycles in relation to fluctuations in differential pressure across the gills, as indicated by the differential pressure waveforms associated with the opercular and buccal components respectively. This is thought to maximize ventilation of the interlamellar gas exchange surfaces throughout the respiratory cycle.

The ratio of mean differential pressure to ventilatory flow is regarded as an indirect measure of changes in gill resistance during each cycle (Hughes & Shelton, 1958; Hughes & Saunders, 1970). Using this method of analysis, gill resistance has been shown to decrease as $\dot{V}_G$ increases over the short term in rainbow trout (Hughes & Shelton, 1958). Other studies (Hughes & Shelton, 1962; Saunders, 1962; Hughes, 1966; Hughes & Morgan, 1973) have reported reductions in utilization with large increases in $\dot{V}_G$ as well, - at least over the short term. From this it was concluded that a drop in total gill resistance must

reflect increases in the porportion of ventilatory flow passing through non-respiratory pathways in the gill sieve rather than through the interlamellar pathways where gas exchange takes place.

In this study gill resistance, as indicated by the $^{MDP}/\dot{V}_G$ ratio, increased with acclimation temperature. It would appear, therefore, that the increase in $\dot{V}_G$ associated with acclimation to higher temperatures may not have been sufficient to force the primary filaments apart, and allow flow through axial pathways. The increase in utilization with increased acclimation temperature closely paralleled that in gill resistance. This suggests that interlamellar flow was, in fact, maintained as suggested above. Thermally-acclimated rainbow trout, therefore, show the ability to increase $\dot{V}_G$ in response to increased oxygen demands while maintaining the integrity of the gill sieve such that ventilatory flow is maximized at exchange surfaces. This fact is supported by the observed increases in gill resistance and utilization with acclimation temperature.

The change in the relative minute volume (RMV) with acclimation temperature is also included in Fig. 37. It is apparent from the equation used to calculate this term ((a + b - c) x VR) that the rate and magnitude of the change in RMV is identical to that of the mean differential pressure. Heath and Hughes (1973) introduced this term as an indirect means of indicating changes in ventilatory flow ($\dot{V}_G$). The relationship between RMV and $\dot{V}_G$ depends on gill resistance to water flow. It appears from the data in this study (Fig. 37) that RMV provides a poor estimate of $\dot{V}_G$, for gill resistance is not constant.

There is a lack of data in the literature pertaining to the effects of temperature on cardiovascular function. From the summary of reported values in Table 5 some trends are perhaps apparent, however, with respect to changes in acclimation temperature. Rainbow trout appear to increase both cardiac

rate and stroke volume in response to increasing acclimation temperature (Table 5). Although no estimates of cardiac output ($\dot{Q}$) were made in this study, rainbow trout did increase heart rate with increasing acclimation temperature. It is probable, assuming the trends shown in Table 5 are real, that the thermally-acclimated trout in this study increased cardiac stroke volume as well. The P-Q and Q-T intervals, the periods of time corresponding to the contraction of the atrium and ventricle respectively, also decreased with increased acclimation temperature (Table 11). The changes in heart rate and electrocardiographic intervals indicate the cardiac rate is extremely sensitive to temperature. Changes in cardiac activity, such as these, could be a result of a direct effect of temperature on the pacemaker cells of the heart, which are restricted to the sino-atrial region in trout as suggested by Randall (1970a). This effect is probably mediated via increases in the ionic conductances of the cardiac muscle membranes with temperature, resulting in increased rythmic activity (Randall, 1970a).

The significant increase in utilization observed in this study indicates that changes in conditions for gas exchange at the gills, are probably involved in the thermo-acclimatory response of resting trout. Randall (1970b) proposed that changes in utilization are related to deadspace phenomena, and divided ventilatory flow qualitatively into three component volumes. These are: (1) respiratory volume, (2) residual volume, and (3) shunted non-respiratory water. The shunted water volume, passed over the gills but not involved in gas exchange, is made up of three deadspace volumes. These are: (1) diffusional deadspace, (2) distributional deadspace, and (3) anatomical deadspace.

Davis and Randall (1973a) have attempted to quantify deadspace phenomena at the gills of rainbow trout using the data of Davis and Cameron (1970) as noted earlier. They found that the total shunted water accounted for only 30% of total ventilatory flow in resting trout over a $\dot{V}_G$ range of 44 to 120

mls min^{-1}. Diffusional and anatomical deadspace together accounted for less than 5% of total $\dot{V}_G$, with distributional deadspace making up the major portion of shunted water. It was concluded that at low to moderate $\dot{V}_G$ values (<300 mls min^{-1}) anatomical and diffusional deadspaces constitute less than 20% of total $\dot{V}_G$. Distributional deadspace, however, decreases with increasing $\dot{V}_G$ due to an increase in the number of secondary lamellae perfused with blood (Davis, 1972; Booth, 1978). The resulting increase in the effective exchange area of the gills, together with the maintenance of respiratory flow as indicated by the constancy of gill resistance serve to maintain utilization in the face of moderate increases in $\dot{V}_G$ at constant temperature. They concluded that deadspace phenomena does not limit gas exchange significantly at normal resting levels of ventilatory flow in rainbow trout (Davis & Randall, unpublished data).

This relationship appears to hold for resting trout at constant temperature in this study as well, for utilization and gill resistance increased in the face of moderate increases in $\dot{V}_G$ from ∿50 to ∿200 mls min^{-1} between 2° and 18°C. It should be pointed out, however, that this thermoacclimatory response may be somewhat different from the acute response to increases in $\dot{V}_G$ by trout at constant temperature in the experiments of Davis and Cameron (1970). It is possible that the characteristics of water and blood involved in diffusion at the gills may change with acclimation temperature. A survey of reported values for the respiratory characteristics of water and blood at different acclimation temperatures, however, does not reveal any obvious trends (Table 4). It appears probable, however, from the values for arterial and venous saturation in Table 4 that over the range of acclimation temperatures employed in this study resting trout are able to fully saturate arterial blood at the gills, and extract sufficient oxygen from blood in the tissues to satisfy oxygen requirements.

Although data is lacking, it is possible that increases in effective

exchange area, may, in fact, also be important in the observed thermoacclimatory change in utilization. Lamellar perfusion in resting trout may increase with acclimation temperature (i.e., at 2°C only 10% of total lamellae are perfused, at 10°C only 30%, and at 18°C only 60%). This type of response could account for the observed increase in utilization, and would still leave the branchial exchanger system sufficient flexibility to respond to immediate increases in oxygen demand at any given acclimation temperature (e.g., the imposed temperature cycle on statically-acclimated fish).

In summary, resting rainbow trout appear to compensate for increases in acclimation temperature by means of adjustments in ventilation, and possibly perfusion and the conditions for diffusion at the gills. Associated increases in water and blood flow rates must be closely regulated so as to limit dead-space phenomena, such that utilization and gill resistance can increase. Although no direct estimate of the $\dot{V}_{G/\dot{Q}}$ is possible (since cardiac output was not measured) the small increases in the cardiac-to-ventilatory rate ratio observed lend some support to this suggestion. However, reported values for $\dot{V}_{G/\dot{Q}}$ (Table 5) give some indication that $\dot{V}_{G/\dot{Q}}$ decreases as acclimation temperature increases. In fact, if it is accepted that cardiac stroke volume increases with acclimation temperature as indicated in Table 5 then the $\dot{V}_{G/\dot{Q}}$ ratio for resting trout in this study would also decrease as acclimation temperature increases. For example, if cardiac stroke volume is assumed to be 0.15, 0.50, and 1.0 mls at 2°, 10°, and 18° C respectively (similar in range to values in Table 5), then the $\dot{V}_{G/\dot{Q}}$ ratios are 10.1, 3.7, and 2.3 respectively. These values correspond well with the values given in Table 5 for resting trout at similar temperatures.

3. Diurnal changes in Cardiovascular-Respiratory Parameters of Fish Acclimated at Constant Temperature.

It is fairly well established that fish exhibit endogenous circadian oscillations in various physiological functions. Schwassmann (1971) has reviewed literature pertaining to biological rhythms in fish. The self-sustained oscillation of the biological clock can be coupled to some degree to exogenous oscillations in abiotic (temperature, photoperiod) or biotic (food organisms) factors. Daily periodicity is expressed in various overt rhythms including activity, and oxygen consumption.

In this study, rainbow trout, thermally-acclimated at constant temperatures (2°, 10° and $18^{\circ}C$) and under a 12/12 light-dark photoperiod regime, however, failed to show any significant diurnal rhythm in the cardiovascular-respiratory parameters observed. The basis of these negative findings is uncertain. Seemingly contradictory evidence of this nature, has been attributed to the inability of the recording methods used to detect small changes in activities under the particular experimental conditions involved (Schwassmann, 1971). In this study, the trout remained quiescent in the respirometer chambers under constant temperature conditions. Since activity was not measured, it is possible that oscillatrons in spontaneous activity were of such small magnitude that they were not detectable in terms of changes in oxygen consumption. On the other hand, if a daily periodicity did in fact exist, it is possible that the relationship between its rhythm and the sampling schedule employed was such that overt changes in rhythm were undetected. The grouping of data did not appear to mask any such evidence as no individuals exhibited periodicity. It should also be noted that the nature of the endogenous biological rhythm and its relationship to these environmental periodicities is poorly understood. It is apparent, however, that the history of the particular species, in terms of genotype and phenotype, may account for some of the intraspecific variation observed in circadian rhythms.

4. Diurnal Changes in Cardiovascular-Respiratory Activities in Response to Imposed Diurnal Temperature Cycle.

Thermal acclimation in teleosts has generally been studied using constant acclimation temperatures. The effect of acute changes in temperature has also been studied using fish acclimated to constant temperature, and then exposed to abrupt, or steady changes in temperature over periods of minutes or hours. Few studies (Heath, 1963; Toews & Hickman, 1969), however, have considered either acute or chronic responses to diurnal cycling temperatures like those occurring in natural freshwater environments. Toews and Hickman (1969), however, examined the effects of diurnally-cycling temperatures on water-electrolyte balance in rainbow trout. In their study trout were exposed to a temperature cycle (between 8° and 18°C) similar to that observed for a trout stream in the foothills of Alberta during the hot summer months. Following an acclimation period of 42 days the cycled fish appeared to have acclimated to the lower portion of the cycle, as indicated by a comparison between ion levels in cycled and statically-acclimated fish at coincident temperatures. One explanation given for this response was that, due to the nature of the temperature cycle, the fish spent more time at the lower temperatures (8° to 10°C). They proposed that in order to demonstrate this point conclusively a "sine wave" temperature cycle should be employed in which the fish would experience equal times at all temperatures.

In another study Heath (1963) compared thermal tolerances in groups of cutthroat trout (Salmo clarki) acclimated to square wave cycling temperatures (between 10° and 20°C) of varying periodicity, and those held under constant acclimation temperature conditions. The cycled fish, in this case, appeared to respond to the highest temperature in the cycle, and the maximum tolerance was obtained in fish also exposed to a natural 24 hour photoperiod regime (12/12 LD).

(i) Effect of diurnally-cycling temperatures ($10^{\circ} \pm 4^{\circ}C$) on rainbow trout statically acclimated to $10^{\circ}C$ and exposed to an initial cycle compared to trout acclimated to cycling temperatures ($10^{\circ} \pm 4^{\circ}C$).

With few exceptions rainbow trout acclimated to cycling temperatures were generally similar to trout statically-acclimated to $10^{\circ}C$, and exposed to the cycle for the first time. The sinusoidal nature of the temperature cycle insured that the fish were equally exposed to the upper portion and lower portion of the cycle. It was felt, therefore, that any thermoacclimatory changes would be obvious in response to a moderate cyclical temperature change of this nature.

Both cyclically-acclimated and statically-acclimated ($10^{\circ}C$) rainbow trout responded to the temperature cycle with marked changes in $\dot{V}_{O_2}$, accompanied by rate changes in the various ventilatory-cardiovascular parameters recorded. For example, trout exhibited changes in overall $\dot{V}_G$ ($Q_{10}>2$), as well as changes in ventilatory rate and stroke volume. The former were slightly greater in the case of cyclically-acclimated fish, while the latter were more pronounced in statically-acclimated fish at the highest temperature in the cycle. Marked variations were also observed in the pressure waveforms of the respiratory pumps. These were more pronounced than cyclic changes in mean differential pressure (MDP). Although MDP did not differ significantly between acclimation groups, the relative contribution of the opercular and buccal differential components varied between individuals within acclimation groups, and between cycled and statically-acclimated trout. At the peak of the cycle ($14^{\circ}C$) the opercular component was slightly greater in cyclically-acclimated fish. The buccal component was generally dominant in statically-acclimated fish, while in cyclically-acclimated fish both components contributed equally to the mean differential pressure. The adaptive significance, if any, of these small changes is not clear. Similar variability in the relative contribution of

of the pumps has been observed by previous workers (Hughes & Roberts, 1970; Hughes & Saunders, 1970; Heath & Hughes, 1973). It appears there are a number of equally effective strategies to meet the heightened ventilatory demands associated with temperature stress (Hughes & Roberts, 1970).

Gill resistance - the ratio of mean differential pressure to $\dot{V}_G$ - exhibited moderate, but somewhat irregular changes in both static and cyclically-acclimated fish in response to the temperature cycle. The absence of a significant reduction in gill resistance with increased $\dot{V}_G$ in the cycled fish, however, indicates that the integrity of the gill sieve was maintained. In addition, the close relationship between gill resistance and utilization indicates that ventilatory flow was maximized over gas exchange surfaces in the face of marked cyclical changes in $\dot{V}_G$.

The cardiovascular system also exhibited some response to diurnal temperature cycles in terms of electrocardio-graphic intervals and heart rate. It is generally held that short-term increases in temperature have a negative ionotropic effect, and positive chronotropic effects on the fish heart (Randall, 1970a). As was indicated earlier, however, changes in temperature are probably associated with changes in venous return which would serve to maintain stroke volume in the face of a negative ionotropic effect of temperature on the heart. (Stevens, et al., 1972).

In the present study, therefore, cyclical changes in peripheral resistance similar to those observed in cardiac rate may have operated to hold stroke volume at a fairly constant level.

As was the case for statically-acclimated fish tested at constant temperature, cyclical changes in utilization indicated that modifications in the conditions for gas exchange at the gills were probably involved in response to cyclying temperatures between 6° and $14^{\circ}C$. The deadspace phenomena (Davis & Randall, 1972) would not be expected to impede gas exchange over the low

range of $\dot{V}_G$'s observed (~80 to ~160 mls min^{-1}). Fluctuations in utilization under cycling temperature conditions may be accountable in terms of changes in effective exchange area brought about by changes in the number of secondary lamellae perfused.

In summary, resting rainbow trout (both statically- and cyclically-acclimated) appear to compensate for diurnal changes in temperature between 6° and 14°C by modifications in ventilation, and possibly gill perfusion and the conditions for diffusion at the gills. The maintenance of the $^{CR}/_{VR}$ ratio lends some support to the suggestion that concurrent adjustments in ventilatory flow and cardiac output were apparently closely regulated to limit deadspace phenomena and maximize gas exchange. However, if it is assumed that cardiac stroke volume remains constant with cycling temperature, and a value of 0.5 mls, as suggested earlier for trout at 10°C, is taken as the value of cardiac stroke volume under cycling conditions, a hypothetical $\dot{V}_{G/\dot{Q}}$ ratio can be calculated as before. The $\dot{V}_{G/\dot{Q}}$ ratio obtained in this way is approximately the same in statically- and cyclically-acclimated trout at 10°C (4.0), and almost identical to that for statically-acclimated fish at constant temperature (10°C). The $\dot{V}_{G/\dot{Q}}$ ratio for trout exposed to cycling temperatures, calculated in this manner, however, would exhibit slight increases with increasing temperature during the cycle. In any case, it would appear that ventilation and perfusion were closely coordinated in response to cycling temperatures between 6° and 14°C.

(ii) Effect of an increase in temperature from 2° to 6°C on rainbow trout acclimated to 2°C.

Rainbow trout statically acclimated at 2°C and exposed to a 4°C change in temperature equivalent to the upper portion of the cycle employed to the other groups exhibited quasi-cyclical responses, similar to those seen in 10°C trout. Although responses were generally less pronounced than at 10°C or 18°C observed increases in the various cardiovascular and respiratory parameters

measured were similar to those of statically- or cyclically-acclimated trout to the upper portion of the cycle between 10° and 14°C. 2°C rainbow trout appeared to compensate for moderate increases in oxygen demand through increases in ventilation and possibly gill perfusion and conditions for gas exchange at the gills. Gill resistance remained fairly constant, which suggests that deadspace phenomena at the gills was reduced. If a cardiac stroke volume of 0.15 mls is assumed, as proposed earlier, and applied to the data for trout in this study, the $\dot{V}_G/\dot{Q}$ ratio would equal 10.1 at 2°C and 11.5 at 6°C. The close agreement between these two ratios adds further support to the suggestion that increases in ventilation and perfusion were likely regulated in response to the temperature cycle in 2°C trout.

(iii) Effect of diurnally cycling temperatures between 14° and 22°C on rainbow trout acclimated to 18°C and exposed to the cycle for the first time.

The response of rainbow trout acclimated to 18°C to the cycle differed in some respects from that of trout acclimated to 10°C or to the 10 $\pm$ 4°C temperature cycle. 18°C- trout responded to the temperature cycle with marked changes in $\dot{V}_{O_2}$; changes similar in magnitude to those seen in 10°C fish. Rate changes in ventilatory and cardiac activities associated with these changes in $\dot{V}_{O_2}$ were somewhat different, however, especially in response to the upper portion of the cycle between 18° and 22°C and will be discussed below. Rainbow trout acclimated to 18°C exhibited marked changes in $\dot{V}_G$ in response to the cycle via adjustments in ventilatory rate and stroke volume. Marked changes in the pressure waveforms and differential pressure were also observed. Although some variation is evident in the data, the buccal and opercular pumps generally contributed equally to the mean differential pressure. These findings are in good agreement with the general ventilatory scheme for teleosts described by Hughes & Shelton (1959) and described earlier. It was evident, however, that respiratory aberrations were occurring in certain individuals at 22°C as

indicated by large fluctuations in the magnitude of the pressure components. Hughes and Roberts (1970) and Heath and Hughes (1973) also observed ventilatory aberrations above 20°C in rainbow trout subjected to acute thermal stress. In these studies the amplitude of the pressure waveforms associated with the buccal and opercular pumps exhibited marked changes at high temperatures (20° - 26°C). In many individuals, pressure in one or other of these components dropped sharply and double reversals became more frequent. Hughes and Roberts (1970) concluded that the respiratory pumps were becoming increasingly uncoupled, and were unable to meet increased ventilatory demands associated with high temperatures. It seems probably that 18°C rainbow trout in this study were exhibiting the first signs of such ventilatory problems at the highest temperatures of the cycle.

The response of the cardiovascular system to cycling temperatures also provided some evidence that these fish were experiencing respiratory difficulties at the higher temperatures in the cycle. Cardiac rate did not increase in response to the upper portion of the temperature cycle. In fact, cardiac rate generally decreased or did not change at 22°C. Small increases in cardiac rate were seen in only a few fish. Bradycardia has been observed under similar temperature conditions (20° to 26°C) in rainbow trout subjected to short-term increases in temperature (Hughes & Roberts, 1970; Heath & Hughes, 1973). The onset of cardiac braking and bradycardia in the studies cited occurred at different temperatures depending on the individual (21.0 to 26.5°C), but generally preceded lethal conditions by 0.5 to 2.0°C (Heath & Hughes, 1973). The findings of the present study correspond well with these values.

It has been proposed (Hughes & Roberts, 1970; Heath & Hughes, 1973; Roberts, 1973) that as temperature increases, oxygen demand and consequently ventilatory metabolic cost may become so prohibitive that ventilation becomes inadequate, and the level of oxygen in the blood drops. Decreases in arterial

blood oxygen content have been observed at high temperatures (20° to 26°C) indicating that the efficiency of the gas exchanger has decreased (Heath & Hughes, 1973). In these studies trout appeared to compensate for decreases in arterial oxygen content by increasing the arterio-venous oxygen difference. This oxygen 'reservoir' was soon depleted, however, since venous oxygen content fell to zero at 23°C.

It is generally felt (see Roberts, 1973; Heath, 1973) that the ventilatory dissynchronization at high temperature results from central nervous system oxygen starvation, so acute that aberrant nervous activity occurs in the brain respiratory centres. The resulting loss of coordination in the respiratory pumps then impedes oxygen transfer from the water to the blood, and further reduces blood oxygen levels. Bradycardia takes place when blood oxygen levels fall to some sufficiently low level such that a condition of internal hypoxia exists (Hughes & Roberts, 1970; Hughes & Saunders, 1970; Heath & Hughes, 1973; Roberts, 1973). Unless reversed, this state of cardiac inhibition continues until death.

The cardiovascular-ventilatory changes observed in 18°C- rainbow trout exposed to cycling temperatures suggests that some individuals were, in fact, experiencing problems of this type at the highest temperatures. The subsequent decrease in temperature may well have prevented the situation from becoming lethal. In two individuals, however, death did occur as temperatures approached 22°C.

Changes in utilization observed in 18°C trout exposed to cycling temperatures were small, and as in the case of cardiac rate and gill resistance, failed to increase in the upper portion of the cycle. It appears, therefore, that the observed adjustments in ventilation (i.e., VR, Vsv), cardiac rate and possibly changes in cardiac output and the conditions for gas exchange at the gills (i.e. lamellar recruitment) were only sufficient to compensate for the changes

in oxygen demand associated with the lower portion of the diurnal temperature cycle (between 18° and 14°C). The fact that utilization did not increase at higher temperatures would suggest, however, that the fish were unable to significantly modify conditions for gas exchange at the gills in response to the upper portion of the cycle (between 18° and 22°C). Although evidence is lacking, the trout must have relied upon changes in water and blood flow-rates to increase oxygen uptake at the highest temperatures in the cycle.

The reason why utilization became rate-limiting at higher temperatures is not obvious, and is difficult to explain because of the complexity of the factors involved. Davis and Randall (1972) found that anatomical and distributional deadspaces remained small at low to moderate levels of ventilatory flow (<300 mls). Distributional deadspace, however, should decrease with increasing $\dot{V}_G$ as the number of secondary lamellae being perfused increases. This increase in effective exchange area together with the maintenance of gill resistance was concluded to be sufficient to maintain utilization, and limit deadspace phenomena with moderate increases in $\dot{V}_G$ at constant temperature (Davis & Randall, 1972).

A similar explanation seems to apply in the case of statically-acclimated 18°C trout upon initial exposure to the upper portion of the temperature cycle. Although evidence is lacking it is possible to speculate as to the possible set of circumstances existing for trout at high temperatures in the cycle as follows. When exposed to the increase in temperature above 18°C, modifications in the conditions for gas exchange at the gills could not be amplified further and utilization levelled off as discussed earlier. Any further increases in oxygen demand, therefore, must have been met by increases in ventilation and cardiac output. At very high temperatures (20° to 22°C) these adjustments also became prohibitive and the arterial blood oxygen content began to decline. The increased oxygen requirements of the tissues must then have been met by

increased utilization at the tissues, by an increase in the arterio-venous oxygen difference with temperature (Heath & Hughes, 1973). This adjustment is somewhat limited in scope, however, as venous oxygen levels would soon become depleted (23°C) and arterial blood oxygen would continue to drop. At some point the low levels of oxygen in the blood would cause ventilatory aberrations to occur decreasing ventilatory efficiency and circulating blood oxygen levels even more. The observed bradycardia was probably invoked in response to a form of internal hypoxia when blood oxygen levels had finally dropped to very low levels in certain individuals in the present study.

The observed response of 18°C- rainbow trout to the upper portion of the temperature cycle indicates that the above set of circumstances probably did exist to varying degrees in certain fish. It appears that some of the 18°C trout at 22°C exhibited the first signs of cardio-ventilatory problems. In general, however, the trout were still able to cope with the situation at high temperatures (18° to 22°C). Gill resistance remained fairly constant with temperature, exhibiting a somewhat similar response to that of utilization, which would suggest that the integrity of the gill sieve was maintained, and that water generally followed respiratory interlamellar pathways, as opposed to nonrespiratory axial pathways. As the temperature was cycled above 18°C, any increases in water and blood flow, therefore, must have been closely regulated so that gill resistance and utilization did not fall. In fact, if it is assumed that cardiac stroke volume for 18°C fish is equal to 1.0 mls and does not change with cycling temperatures, as proposed earlier, the calculated $\dot{V}_G/\dot{Q}$ ratio is approximately 2.5 at 18°C, and 3.5 at 22°C. Although the increase in the hypothetical $\dot{V}_G/\dot{Q}$ here is greater than that observed for trout cycled between 6° and 14°C, it still remains fairly constant with temperature, such that deadspace phenomena would be reduced. In summary, therefore, as cycling temperatures approach 22°C the major rate

limiting factor may well be the diffusion resistance of the gills in relation to the reduced oxygen levels in the water.

(iv) Speculations on thermal acclimation with cycling temperatures.

The fact that cyclically- and statically-acclimated rainbow trout exhibited virtually identical responses to a diurnal temperature cycle between 6° and 14°C warrants further discussion. Two possible explanations exist for these observations. Either both groups of fish acclimated to the cycle, or neither group exhibited thermal acclimation to the cycle. In the case of the first hypothesis, for this to be true extremely fast rates of acclimation would be required. Fry (1971), in reviewing the rates of thermal acclimation for various species, quotes values as low as 1°C day^{-1} for goldfish, and as high as 1°C $hour^{-1}$ for various salmonids. In the present study temperature changed in a sinusoidal fashion between 6° and 14°C. This meant that the rate of change was always less than 1°C $hour^{-1}$, and that the rate decreased as the temperature was cycled away from the midpoint. Almost equal time was spent at temperatures near the extremes (13° - 14°C, or 6° - 7°C; six hours; rate of change $\sim$0.33°C $hour^{-1}$) as was spent at all intermediate temperatures combined (7° - 13°C; six hours; rate of change $\sim$0.80°C $hour^{-1}$).

The nature of the temperature cycle, therefore, may have played a part in allowing thermal acclimation to occur in the case of the statically-acclimated trout (10°C).

The second hypothesis, that acclimation did not occur after short- or long-term exposure to the cycle is less easily rationalized, especially in the latter case. This could mean, however, that the oxygen transport system in rainbow trout possesses sufficient capacity to cope with the moderate cyclic changes in temperature between 6° and 14°C under the conditions of the experiment. Thermal acclimation in this sense cannot be defined as static or cyclic, but rather that the fish are acclimated in a broader sense to a

moderate thermal range. The precise response to moderate changes in temperature within this range may involve short term adaptive changes in the various physiological rate functions. If this is true, both the statically- and cyclically-acclimated trout could be considered to be acclimated to an equivalent thermal range.

Evidence for this can be adduced from a comparison of the response to the cycle for both cycled- and statically-acclimated fish and the static response at 10°C. In general, the midpoint values for cyclic responses corresponded well with the values at constant temperature (10°C). The cyclic values did not shift upwards or downwards relative to the static acclimation values at 10°C. In the case of statically-acclimated fish this could mean that the fish were acclimated to 10°C, and were simply responding to temperature changes well within their physiological capabilities. The cyclically-acclimated fish, however, exhibited similar responses, and as was pointed out earlier, the nature of the cycle meant that very little time was spent at 10°C, the midpoint of the cycle. For the particular cycle used here, therefore, the time at any given temperature may not be the determining factor in acclimation. The real significance for the cyclic response may be in the magnitude of the temperature change. If the fish are simply responding to the temperatures within some thermo-acclimatory range, then a symmetrical cyclic response would be expected. This is not to say that thermal acclimation is not important in the response to cycling temperatures, for its role is apparent at temperatures approaching the limits of the thermal tolerance range.

In general, thermal acclimation would not be expected to play any significant role in response to cycling temperatures until changes in the cardio-vascular-respiratory rate functions become prohibitive. It would have been interesting, therefore, to have observed the effects of larger temperature cycles which would approach physiological limits. In addition, it would be

worthwhile, to see if rainbow trout acclimated for several weeks to a temperature cycle between 14° and 22°C exhibited a different response to that observed for trout acclimated to 18°C in the present study.

CONCLUSIONS

SUMMARY

A. For rainbow trout thermally-acclimated and observed at constant temperatures.

1. To analyse the effects of thermal acclimation on ventilatory and to a lesser extent cardiovascular function under constant temperature conditions values were obtained for various physiological rate functions ($\dot{V}_{O_2}$, VR, Vsv, $\dot{V}_G$, CR %U, MDP, etc.) for rainbow trout acclimated to 2°, 10° and 18°C. The $^{CR}/_{VR}$ ratio and gill resistance ($^{MDP}/_{\dot{V}_G}$) were then calculated.

2. Rainbow trout exhibited a marked increase in $\dot{V}_{O_2}$ with acclimation temperature. This increase in oxygen uptake appears to have been achieved by means of coincident rate changes in a number of ventilatory and to some extent cardiac activities. Trout exhibited increases in ventilatory rate (VR), and stroke volume (Vsv) resulting in increased ventilatory flow ($\dot{V}_G$) with acclimation temperature. In addition, marked increases in cardiac rate were observed with increased acclimation temperature as well. This data suggests that rainbow trout respond to the conditions of reduced oxygen availability associated with increased acclimation temperature by adjustment of ventilatory activity involved in convective oxygen transport to the gills. Similar conclusions appear to apply for convective transport by the blood to the tissues, although measurements of cardiac output ($\dot{Q}$) were not made.

3. Thermally-acclimated rainbow trout increased mean differential pressure (MDP) with acclimation temperature. Gill resistance (GR), as indicated by the ratio of MDP to $\dot{V}_G$, also increased with temperature. It would appear from the increase in gill resistance, therefore, that the observed increase in $\dot{V}_G$, ($\sim$50 to $\sim$ 200 mls min^{-1} between 2° and 18°C) was not associated with significant amounts of non-respiratory flow through axial pathways in the gills. The observed increase in utilization (%U) lends strong support to this suggestion.

4. Significant increases in utilization (%U) were observed for thermally-

acclimated rainbow trout with increased acclimation temperature. This finding suggests that thermal acclimation to higher temperatures probably involves changes in conditions for gas exchange at the gills. The reasons behind the observed increase in diffusive transport are unknown, due to the inherent complexity of this measurement. A possible explanation is that %U changed in response to an increase in effective exchange area via an increase in the number of secondary lamellae perfused.

5. There was no evidence of any significant diurnal rhythm in any of the cardiovascular-respiratory parameters observed for rainbow trout thermally-acclimated at constant temperatures (2°, 10°, and 18°C) and under a 12/12 light-dark photoperiod regime.

B. For rainbow trout exposed to diurnally cycling temperatures.

1. To evaluate the influence of cycling temperatures in terms of an immediate, as opposed to acclimatory response, various ventilatory-cardiovascular rate functions ($\dot{V}_{O_2}$, Vsv, VR, $\dot{V}_G$, CR, %U ...etc.) were observed for rainbow trout either acclimated to cycling temperatures (10° $\pm$ 4°C) or acclimated to constant temperatures (2°, 10°, & 18°C) and exposed to the cycle for the first time (for 10°C-trout, 10° $\pm$ 4°C; for 18°C- trout, 18° $\pm$ 4°C). Fish acclimated to 2°C were also exposed to a change in temperature between 2° and 6°C equivalent to the upper portion of the diurnal cycle employed above.

2. With few exceptions the response of trout acclimated to cycling temperatures was generally similar to that of trout acclimated to constant temperatures and exposed to the cycle for the first time.

3. Rainbow trout of all groups exhibited significant changes in oxygen consumption ($\dot{V}_{O_2}$) with cycling temperatures. As was the case under static conditions, changes in $\dot{V}_{O_2}$ appeared to be largely mediated by adjustments in ventilatory flow ($\dot{V}_G$) associated with changes in rate (VR) and stroke volume (Vsv). With the exception of 18°C trout at high temperature, marked changes were also

observed for cardiac rate. The data from these experiments suggest that rainbow trout respond to changes in oxygen demand associated with cycling temperatures by modification of ventilatory activities involved in convective oxygen transport by water. Although data is lacking, it appears that changes in convection by the blood may also be involved in this response.

3. Rainbow trout thermally-acclimated to 18°C and exposed to an initial diurnal temperature cycle exhibited a different response than was observed for trout in other acclimation groups. Although 18°C- trout exhibited marked increases in $\dot{V}_G$ in response to the peak of the cycle, aberrant ventilatory activity was observed for some individuals at higher temperatures. In addition, heart rate either failed to increase or decreased relative to midpoint values in almost all individuals. This evidence suggests that adjustments in ventilation at high temperatures were insufficient to meet oxygen demands. As temperature increased, oxygen demand may have become so prohibitive that ventilation became inadequate and the level of oxygen in the blood decreased. In view of the ever increasing demands for oxygen with temperature, this would eventually lead to a condition of internal hypoxia causing ventilatory dissynchronization and bradycardia as observed for these fish.

4. Mean differential pressure (MDP) exhibited significant changes with cycling temperatures for all acclimation groups. Gill resistance also showed significant variation with cycling temperatures (i.e., increased and decreased with temperature) for all groups, with the exception of 18°C- trout at high temperatures. In the latter case gill resistance did not increase in response to the peak of the temperature cycle. The fact that gill resistance did not decrease with increasing temperature suggests that rainbow trout were able to maintain the integrity of the gill sieve in the face of significant changes in $\dot{V}_G$ associated with cycling temperatures. As was suggested for static conditions, therefore, deadspace phenomena appears to be closely regulated under the cyclic

temperature conditions employed in this study. A suggestion, further supported by the observed maintenance of utilization under cycling conditions as well.

5. Significant changes in utilization were observed for trout exposed to diurnally cycling temperatures. With the exception of 18°C- trout, %U generally increased and decreased with the temperatures of the cycle. In the former case, however, as was observed for gill resistance, %U failed to increase in response to the highest temperatures in the cycle. This data suggests, as was the case for static conditions, that the response of trout to cycling temperatures involves changes in the conditions for gas exchange at the gills. The manner in which these changes in diffusive transport are achieved, although unknown, may involve changes in effective exchange area at the gills. The response of 18°C- trout, in terms of %U, indicates that diffusive oxygen transport at the gills may have become rate-limiting with respect to the increased oxygen requirements associated with high temperatures. These fish may have been unable to further modify the conditions for diffusion (e.g, effective exchange area) above 18°C. This would place additional loads on the ventilatory and cardiovascular systems which may have resulted in the aberrant responses observed at higher temperatures.

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APPENDICES

Appendix A. Cannulation Procedure.

Polyethylene cannulae were implanted in the buccal and cleithral chambers. For buccal catheterization a hollow 1/8" stainless steel probe, bevelled and sharpened at one end, was forced through the buccal opening from the outside. A P.E. 160 catheter, heat flared at one end, was then passed through the probe from the outside of the mouth, after which the probe was removed. A polyethylene sleeve (P.E. 240) 1" in length and heat flared at one end, was then passed over the free end of the P.E. 160 catheter and secured by a wound clip. One buccal catheter was installed per fish. By turning the appropriate stop cock water could be sampled or buccal pressure recorded. For cleithral catheterization two small incisions were made, one just anterior to the cleithral bone inside the cleithral cavity, and another just posterior to the cleithral bone in the body wall. The steel probe was then inserted through the incisions in a posterior-anterior direction, and the catheter was inserted as above. The procedure used was similar to that used for buccal catheterization with the following exceptions: (1) a stainless steel spatulae was used to protect the gill lamellae from being damaged; (2) the P.E. 240 sleeves were 2½" in length, and held in place by 2 wound clips and a suture; (3) cannulae were fitted with a large ¼" diameter flare which was contoured and angled to better fit the opercular cavity. Two cleithral cannulae were installed per fish which allowed the simultaneous sampling of post-branchial water and recording of opercular pressure.

Appendix B. Determination of Oxygen Consumption.

Dissolved oxygen was determined by means of the unmodified Winkler method in accordance with the American Public Health Association-approved methods (A.P.H.A.; 1971). Water samples were collected in narrow-mouthed glass bottles with tapered, ground glass, pointed stoppers and flared mouths. 30 ml capacity bottles were used for collecting water samples from the inflow and outflow

water of the respirometer chambers, and 15 ml capacity bottles were used for sampling water from the buccal and opercular catheters.

Large bore tygon tubing used for sampling inflow and outflow water was placed in the bottom of the sample bottles, as was the polyethylene (P.E. 160) cannulae used for sampling from the buccal and opercular chambers. Sample bottles were immersed in a small water bath supplied with water from the upper respirometer tank to minimize temperature changes with sampling. Prior to sampling care was taken that the bottles were overflowing, and air bubbles were not present. Dissolved oxygen was determined immediately after the sample was taken. The iodometric technique used was as follows:

(a) For 30 ml capacity bottles.

(i) collect sample

(ii) add 0.2 ml manganous sulfate solution (Hach)

(iii) add 0.2 ml alkaline iodide reagent (Hach)

(iv) stopper bottle, invert 15 times, allow some settling then invert 10 times.

(v) when approximately 10 mls clear supernatant showing, add 0.2 mls conc. H_2SO_4

(vi) stopper, invert until dissolution complete and an even yellow mixture present.

(b) For 15 ml capacity bottles.

(i) iodometric procedure similar to (a) except that 0.1 mls of manganous sulfate solution, alkaline iodide reagent, and conc H_2SO_4 was used.

The liberated iodine was then titrated with a standard phenylarsine oxide solution (.025N). The titration end-point was detected visually, using a starch indicator. The titration procedure was as follows:

(a) For 30 ml capacity bottles.

(i) pipette 20.2 ml of sample into 30 ml beaker

(ii) add 2 drops of standard indicator solution.

(b) For 15 ml capacity bottles.

(i) pipette 10.1 ml of sample into 30 ml beaker

(ii) add 1 drop of starch indicator solution.

Appendix C. Detailed Analysis of Pressure Waveforms.

(a) area mean pressures for the stages of the respiratory cycle.

(i) opercular stage

$$\text{opercular pressure} = \frac{(\text{area under curve}) \times \text{ventilatory rate}}{\text{time}}$$

+ ve or - ve

= arbitrary units

(ii) Buccal stage

$$\text{Buccal pressure} = \frac{(\text{area under curve}) \times \text{ventilation rate}}{\text{time}}$$

+ ve or - ve

= arbitrary units

(b) area mean differential pressure components

(i) component (a): area under the differential pressure curve attributed to opercular pressure.

$$(a) = \frac{(\text{area under curve}) \times \text{ventilation rate}}{\text{time}}$$

= arbitrary units

(ii) component (b): area under the differential pressure curve attributed to buccal pressure.

$$(b) = \frac{(\text{area under curve}) \times \text{ventilation rate}}{\text{time}}$$

= arbitrary units

(iii) component (c): area under the differential curve attributed to the transitional stage where the sign of the pressure changes from + ve to - ve.

$$(c) = \frac{(\text{area under curve}) \times \text{ventilation rate}}{\text{time}}$$

(c) area mean differential pressure

$$MDP = \frac{(a + b - c)VR}{(time)} \text{ arbitrary units.}$$

Appendix D. Derived Ventilatory Equations.

(a) oxygen consumption

$$\dot{V}o_2 = \frac{\text{flow} \times (\bar{Y}_1 - \bar{Y}_2)}{wt} = \text{mg/kg/hr.}$$

$\bar{Y}_1 - \bar{Y}_2$: mean difference in dissolved oxygen between inflow and outflow water of the respirometer chamber (mg/l).

flow: flow rate through chamber (l/hr)

wt: weight of fish in kilograms.

(b) percent utilization

$$\%U = \frac{P_{insp} - P_{exp}}{P_{insp}} \times 100 = \%$$

P_{insp}: dissolved oxygen of inspired water

P_{exp}: dissolved oxygen of expired water

(c) Ventilatory flow

(i) minute volume

$$\dot{V}g = \text{flow} \times \frac{(\bar{Y}_1 - \bar{Y}_2)}{\frac{P_{insp} - P_{exp}}{wt}} = \text{l/kg/min}$$

(ii) relative minute volume

RMV = (a + b - c) x ventilatory rate, arbitrary units.

(a + b - c): algebraic sum of area mean differential components beneath the differential curve.

(d) Stroke volume

$$Vsv = \frac{\text{minute volume}}{\text{ventilation rate}} = \text{mls}$$

(e) Gill resistance

$$GR = \frac{\text{area mean differential pressure}}{\text{minute volume}} = \text{ratio}$$

Appendix E. Analysis of the Effect of Temperature on Cardiovascular-Respiratory Parameters.

In order to examine the effect of temperature on the rates of the various parameters that were measured, Q_{10} values were calculated using the following formula.

$$Q_{10} = \left(\frac{V_1}{V_2}\right)^{10/(t_1 - t_2)}$$

Q_{10}: factor by which a reaction velocity (or rate function) is increased for an increase in temperature of 10°C.

The Q_{10} values calculated reflect the effect of temperature on the various physiological functions observed. A Q_{10} value of 2 indicates that the rate function doubles with a 10 C° increase in temperature. Q_{10} values vary with temperature being greater in low temperature ranges than in high temperature ranges.

In the case of the interval analysis of the electrocardiogram traces it was necessary to make V_1 and V_2 equal to the reciprocal of the time interval. This modification was necessary, since although heart rate increased, the intervals of the ECG decreased with increasing temperature. The normal Q_{10} analysis would, therefore, have yielded Q_{10} values which were less than one.

Thermal coefficients were calculated to evaluate the effect of temperature on parameters not involving rates. The formulae used was the same as that for Q_{10} values except that V_1 and V_2 represent the magnitude of a given parameter. The thermal coefficient is the factor by which the magnitude of a parameter is changed for an increase in temperature of 10°C.

Appendix:table 1. Analysis of variance for selected samples of cardiovascular and respiratory parameters.

Parameter	Samples selected for comparison of variance **																																															
	1-8	1-10	1-22	2-29	2-33	3-29	3-33	5-35	6-15	6-29	6-33	7-8	8-15	8-29	8-33	10-19	10-28	10-30	12-13	14-15	14-17	14-24	14-31	14-32	15-16	15-32	15-34	15-35	17-21	17-24	17-25	17-26	17-27	17-28	17-31	17-35	21-24	21-30	22-24	22-30	23-24	25-26	26-35	29-30	30-31	32-33	32-34	33-36
$\dot{V}_{O_2}$	*	*	*	*	*	*	*	*	*	*	*	*			*	*			*	*	*	*	*		*	*	*	*	*		*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*
% U	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*		*	*	*	*	*	*		*	*	*			*	*	*	*	*	*	*	*	*	*	*	*
Rel. Min. Vol.	*	*	*	*	*	*	*	*	*	*	*	*				*		*	*	*	*	*	*		*	*	*	*	*		*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*
$\dot{V}_G$	*	*	*	*	*	*	*	*	*	*	*	*				*			*	*	*	*	*		*	*	*	*	*		*	*	*	*			*	*	*	*	*	*	*	*	*	*	*	*
Vsv		*	*	*	*	*	*	*	*	*	*					*			*	*	*		*			*		*				*		*		*		*		*			*	*	*	*		*
Gill Resistance	*	*	*	*	*		*	*								*		*		*			*		*			*				*						*		*					*			
Max. buc. press.	*	*	*	*	*			*	*			*	*			*			*	*	*		*		*	*	*	*				*		*				*		*	*		*	*	*	*	*	*
Max. buc. area	*	*	*					*				*	*	*	*	*		*	*	*	*				*	*	*					*		*			*	*	*	*	*		*	*	*	*	*	*
Min. buc. press.	*		*					*	*			*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*		*	*	*		*	*	*	*	*	*	*			*	*	*	*	*
Min. buc. area	*		*	*		*	*	*	*	*	*	*	*	*	*	*			*	*	*		*		*	*	*	*	*		*	*	*			*	*	*	*	*	*		*	*	*	*	*	*
Max. operc. press.	*	*	*					*	*			*	*			*			*	*		*	*	*	*	*	*	*			*				*	*		*		*					*	*	*	*
Max. operc. area	*	*	*					*	*				*	*	*	*			*	*	*		*	*	*	*	*	*	*		*							*		*					*	*	*	*
Min. operc. press.	*		*			*			*			*	*			*	*			*		*	*	*		*	*				*	*											*					*
Min. operc. area	*		*						*				*	*	*	*				*			*	*	*	*	*	*			*							*		*								*
Area mean diff.pr.	*	*	*	*	*	*	*	*	*	*	*	*	*			*		*	*	*	*	*	*		*	*	*	*	*		*		*	*			*	*	*	*	*	*	*	*	*	*	*	*
Operc. comp. a	*	*	*					*				*											*					*							*	*									*			
Buccal comp. b		*	*	*	*			*	*	*	*	*				*			*	*	*		*		*	*		*						*				*		*	*			*	*	*		
Reversal comp. c								*					*				*	*					*	*		*	*	*				*	*	*	*	*					*			*	*		*	*
Ventilation rate	*	*	*	*	*	*	*	*	*	*	*	*				*			*	*	*	*	*		*	*	*	*	*		*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*
Cardiac rate	*	*	*	*	*	*	*	*	*	*	*	*				*			*	*	*	*	*	*	*	*	*	*	*		*			*			*	*	*	*	*			*	*	*	*	*
Card./vent. rate		*	*		*	*	*		*	*	*															*				*		*	*															
P-Q interval	*	*	*	*		*		*				*				*					*	*				*		*	*	*	*	*	*		*	*		*		*		*	*	*	*	*		*
Q-S interval		*	*						*											*			*	*			*			*				*		*		*		*			*					
S-T interval	*	*	*	*	*	*	*	*	*	*	*	*				*			*	*	*	*	*		*	*	*	*	*		*	*	*				*	*	*	*	*			*	*	*	*	*
R amplitude						*										*																																
Cough rate		*														*	*	*						*		*	*							*				*		*			*					*
Weight	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*														*	*	*			*	*	*	*		*	*	*	*			*	*	*	*
Length	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*						*							*	*	*	*	*	*	*	*	*		*	*	*	*			*	*	*	*
Temperature	*	*	*	*	*	*	*	*	*	*	*	*				*			*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*

* significantly different at the 5 % level of significance
**see appendix table 1a for key to samples selected above.

Appendix table 1a. Key to appendix table 1.

Acclimation Regime	Time (hours) 0	6	12	18	24	30	36	42	48
Static									
2°C	1	2	3	4	5	6	7	8	9
10°C	10	11	12	13	14	15	16	17	18
18°C	19	20	21	22	23	24	25	26	27
Cycling									
10°C ±4°C	28	29	30	31	32	33	34	35	36

Appendix: Table 2 Raw Data Oxygen Consumption (mg/kg/hr)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	18.6	----	19.7	18.4	19.7	17.7	21.6	30.3	25.0
2	16.1	16.1	14.9	20.6	18.5	18.1	10.2	31.4	18.1
3	17.4	13.5	12.7	12.4	11.2	12.4	15.4	31.2	----
4	----	11.8	10.5	16.0	19.0	21.1	14.7	27.8	16.9
5	23.7	27.6	19.7	20.3	25.7	20.4	19.5	41.4	18.7
6	15.5	11.6	8.5	17.0	13.9	11.2	13.0	24.0	17.0
7	20.7	26.0	23.4	21.8	24.1	11.5	18.4	32.2	10.3
8	19.2	25.0	27.1	30.0	22.1	19.2	25.0	37.5	21.7
9	26.7	32.1	16.4	20.6	19.3	24.7	19.7	39.5	27.5
10	14.8	12.4	11.8	16.1	19.6	20.4	15.2	37.0	20.1
Mean	19.2	19.6	16.5	19.3	19.1	17.2	18.1	33.2	19.5
M_1*	22.2	25.7	20.7	22.7	22.0	20.7	21.8	37.2	23.3
M_2**	16.2	13.4	12.3	15.9	16.2	13.8	14.5	29.3	15.6

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	63.6	60.5	69.0	67.3	65.9	39.5	70.0	97.3	68.2
2	55.1	50.0	52.4	55.1	52.8	32.4	48.7	80.6	59.8
3	70.5	65.7	72.1	69.2	75.6	48.1	72.4	99.4	77.9
4	----	54.3	55.5	55.0	50.8	35.3	53.4	80.9	56.5
5	65.3	63.8	63.3	65.8	60.3	41.6	60.9	88.7	56.2
6	69.7	67.6	67.4	68.8	69.6	46.1	66.9	96.3	62.3
7	41.1	39.3	40.9	40.9	41.1	25.6	38.5	74.4	50.1
8	48.9	46.3	49.7	50.6	48.4	29.4	44.3	78.5	52.9
9	61.3	57.4	60.8	61.1	65.2	40.7	58.5	102.3	71.8
10	60.3	59.3	68.1	70.3	71.3	----	66.2	101.9	74.5
Mean	60.5	56.7	60.1	60.7	61.2	36.9	58.2	90.1	63.0
M_1*	68.4	65.2	67.0	67.4	69.0	43.6	66.4	97.7	69.9
M_2**	52.7	51.2	53.2	53.9	53.4	30.1	49.9	82.4	56.2

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	115.3	110.3	115.3	116.9	120.1	88.2	106.7	164.4	124.8
2	125.0	114.8	123.3	127.1	124.6	90.6	129.8	150.5	126.7
3	138.3	128.3	131.7	136.0	132.1	92.6	128.9	196.2	136.0
4	110.0	113.2	113.8	118.6	126.8	80.0	114.8	146.5	123.7
5	121.2	124.3	133.4	128.7	130.8	107.7	140.2	173.3	130.7
6	101.2	99.8	106.8	105.0	107.1	74.7	89.0	141.3	94.3
7	109.7	116.3	111.4	110.0	108.0	82.6	104.3	-----	----
8	122.4	120.4	125.8	128.6	126.9	99.6	121.1	177.2	121.2
9	127.7	125.4	117.2	123.2	118.6	84.8	127.2	185.5	110.4
10	135.5	138.5	134.0	137.6	134.0	96.6	138.3	-----	-----
Mean	120.6	119.1	121.2	122.2	120.1	90.3	121.3	165.4	125.5
M_1*	129.0	126.8	128.2	129.0	131.6	97.2	133.7	180.9	129.4
M_2**	112.2	111.4	114.1	115.4	116.6	83.5	109.9	149.2	111.6

Cycle 10°± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	54.6	44.8	54.2	73.4	57.4	36.8	50.2	75.2	48.9
2	59.2	29.2	45.5	80.7	55.6	37.9	55.9	75.1	64.8
3	50.8	30.2	48.2	85.3	64.0	36.4	55.8	94.6	67.9
4	60.9	39.2	56.3	88.3	58.0	33.1	53.5	94.4	----
5	64.9	40.5	60.1	79.1	60.0	40.1	52.0	77.8	64.7
6	73.3	37.8	58.6	89.0	58.4	35.1	59.7	87.9	66.4
7	75.1	45.6	58.3	106.8	80.1	46.6	64.5	102.4	77.9
8	55.1	32.3	61.4	92.5	78.5	30.7	43.4	-----	65.6
9	68.3	40.8	63.4	94.8	61.4	40.1	71.2	90.5	73.1
10	60.4	44.5	55.2	80.5	56.5	48.7	67.1	93.2	72.8
Mean	62.3	38.5	56.1	87.1	63.0	38.8	57.3	86.7	65.7
M_1*	68.0	42.8	60.2	94.0	69.4	42.6	63.4	94.7	71.1
M_2**	56.5	34.2	52.1	80.3	56.6	34.7	51.3	78.8	60.3

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 3 Raw Data Utilization (%)

8°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	15.3	17.5	12.1	15.4	17.6	19.1	16.9	20.6	17.1
2	19.1	----	15.1	19.8	19.3	18.3	19.0	25.0	----
3	20.0	17.2	10.1	16.0	15.7	17.9	16.8	23.8	18.9
4	----	20.8	19.4	24.8	24.5	20.4	24.5	29.9	22.2
5	18.8	15.7	13.2	14.5	13.5	10.8	11.3	19.8	13.1
6	15.8	17.2	14.5	15.4	17.0	9.2	10.6	20.1	16.3
7	26.8	29.5	25.9	26.7	26.8	25.4	28.4	----	24.4
8	20.3	16.7	17.3	19.4	17.6	----	16.1	20.9	17.8
9	29.7	27.9	23.0	25.3	27.9	26.2	23.9	28.1	25.0
10	19.3	17.4	15.0	16.5	----	18.3	16.5	26.1	14.0
Mean	18.6	19.4	17.4	19.4	19.9	18.4	18.4	24.3	18.8
M_1*	21.9	23.3	20.5	23.7	23.9	20.8	20.5	26.9	22.1
M_2**	15.4	15.0	14.3	16.1	15.8	14.0	14.3	21.6	15.5

12°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	41.9	41.3	41.5	44.0	43.2	27.2	40.4	51.6	46.1
2	35.6	36.7	36.4	37.2	34.7	21.6	30.3	43.4	39.5
3	47.4	44.1	47.0	45.6	47.3	33.8	45.5	59.4	48.1
4	43.9	35.7	40.5	38.3	37.8	27.4	37.6	56.6	36.7
5	43.4	46.2	43.9	42.7	41.7	25.8	42.5	52.7	42.6
6	44.1	43.5	42.9	44.2	44.1	29.8	42.0	57.1	46.5
7	34.8	37.1	35.4	38.2	34.1	27.2	32.8	46.1	40.6
8	44.2	45.2	46.5	42.3	44.7	38.1	40.8	53.7	46.6
9	39.7	41.5	40.8	38.2	42.3	34.2	38.1	45.6	42.1
10	46.3	40.0	48.7	46.8	46.0	----	40.2	54.5	47.6
Mean	40.1	41.9	43.4	41.8	41.6	29.3	39.8	52.1	43.6
M_1*	45.1	45.0	45.5	44.3	45.0	33.2	43.2	55.9	46.4
M_2**	39.1	38.9	39.2	39.3	38.3	25.2	36.4	48.2	40.9

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	50.7	51.7	53.2	55.3	56.1	39.3	47.9	58.7	51.9
2	58.7	60.3	63.7	61.3	60.0	50.5	56.3	51.0	57.6
3	60.5	61.9	54.7	57.3	55.5	46.3	53.8	72.1	55.1
4	63.7	63.3	55.8	58.6	63.2	57.5	57.6	54.5	55.7
5	55.2	55.6	53.6	54.9	60.9	54.4	56.2	67.7	65.1
6	47.9	49.7	55.2	50.3	50.6	44.9	45.3	56.7	47.5
7	52.6	53.3	51.7	51.4	50.3	48.6	55.7	----	----
8	57.6	58.4	59.8	62.2	60.5	53.6	57.6	68.9	60.8
9	54.9	54.5	55.0	56.8	57.3	52.4	54.9	60.7	60.5
10	47.1	51.0	49.4	49.5	52.9	41.7	45.3	----	----
Mean	54.9	56.0	55.2	56.1	57.1	48.6	52.5	61.2	57.2
M_1*	58.8	59.4	58.1	59.5	60.3	52.4	55.7	67.5	61.4
M_2**	51.0	52.6	52.3	52.6	53.9	44.7	49.2	54.9	52.4

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	39.9	36.2	34.8	43.7	40.5	27.4	39.7	45.0	38.7
2	39.1	25.5	32.9	46.4	39.3	----	36.9	41.8	43.5
3	36.7	28.0	30.3	51.6	40.0	30.1	42.1	40.1	38.3
4	41.6	30.4	38.8	49.7	44.4	31.0	40.2	48.4	45.3
5	46.0	36.5	41.0	47.8	45.0	41.0	43.5	50.4	44.2
6	50.9	29.6	38.1	57.2	41.2	29.5	40.8	51.6	45.3
7	50.7	34.7	----	54.9	45.5	35.5	46.1	53.4	45.9
8	39.0	29.2	44.0	59.8	52.3	28.0	34.0	46.5	40.8
9	----	31.8	40.5	49.7	38.7	31.8	40.0	50.9	46.7
10	46.0	39.3	42.2	52.1	47.1	40.1	48.1	50.8	51.9
Mean	43.3	30.3	38.3	51.5	43.4	33.8	41.3	50.1	45.2
M_1*	47.4	35.4	41.5	54.8	46.3	37.8	44.3	52.1	48.2
M_2**	39.3	29.2	35.1	47.8	40.5	29.9	38.4	47.1	41.9

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 4 Raw Data Minute Volume (mls/min)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	60.4	60.6	81.3	55.0	56.3	36.7	64.5	81.1	77.5
2	39.1	43.7	46.5	49.6	45.5	46.5	25.2	67.2	65.8
3	42.2	38.4	34.2	37.2	34.5	32.9	44.8	69.0	31.7
4	37.1	27.1	25.8	30.7	37.0	47.3	28.4	56.7	36.6
5	88.8	86.6	71.8	66.4	91.5	92.9	82.8	111.7	70.8
6	55.6	46.3	28.8	54.7	40.7	56.6	54.2	64.6	52.6
7	40.3	44.7	45.9	42.2	47.2	23.9	33.7	55.4	23.2
8	45.8	73.2	74.5	72.6	61.6	58.7	76.9	88.6	60.2
9	62.5	54.3	34.5	40.3	33.2	50.0	39.3	76.7	55.3
10	36.2	34.2	37.3	45.6	55.4	53.2	43.1	71.4	64.6
Mean	50.8	50.9	48.1	49.6	50.3	49.9	49.3	74.2	53.8
H_1*	63.6	63.9	62.6	58.9	62.7	63.2	63.5	86.2	66.6
H_2**	39.0	37.9	33.5	40.4	37.8	36.5	35.1	62.3	41.0

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	254.2	247.9	249.3	246.6	244.8	186.8	257.6	294.9	255.3
2	197.5	176.8	185.0	190.1	184.6	146.8	195.6	286.1	220.1
3	217.2	195.8	230.4	220.2	228.6	164.4	207.9	303.1	238.1
4	213.9	222.9	207.1	210.3	228.8	170.5	219.2	301.0	247.0
5	226.0	221.1	253.9	240.5	237.9	189.4	253.6	311.9	219.4
6	215.7	206.4	196.4	209.0	206.0	151.6	197.3	304.0	198.4
7	208.7	218.8	219.3	215.2	215.2	172.2	191.7	-----	-----
8	167.7	161.5	170.9	155.1	159.5	130.7	158.7	200.7	165.0
9	223.4	219.1	203.8	204.7	198.9	150.1	207.0	269.0	179.5
10	247.2	223.9	221.4	223.7	210.1	156.1	239.4	-----	-----
Mean	217.2	209.4	213.8	211.7	211.4	161.1	218.7	284.4	211.0
H_1*	234.5	227.5	232.7	230.1	229.9	175.4	237.2	345.9	239.8
H_2**	199.8	191.3	194.8	193.3	192.9	146.8	191.2	267.9	195.7

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	95.8	92.1	101.4	102.8	101.3	82.0	101.3	132.1	94.0
2	105.9	107.2	108.5	112.4	112.7	87.7	108.6	166.2	114.0
3	129.1	128.8	137.9	135.9	143.2	94.5	133.9	179.8	150.4
4	-----	113.4	102.0	99.6	110.7	78.5	102.5	136.4	111.3
5	119.5	113.6	116.3	116.9	121.3	88.4	108.9	150.2	99.3
6	121.0	117.8	118.4	117.9	121.3	91.4	116.4	157.1	104.1
7	94.0	84.7	96.5	91.5	95.2	65.6	94.6	156.3	100.3
8	83.1	75.1	81.5	91.0	80.6	38.8	82.9	146.9	81.5
9	107.1	103.7	103.7	111.2	107.6	79.0	105.3	162.0	120.3
10	125.0	120.1	113.8	124.4	128.0	----	119.5	172.8	129.4
Mean	110.1	105.8	108.0	109.4	112.2	78.4	107.4	156.0	110.5
H_1*	122.3	117.2	118.8	118.4	124.8	91.6	117.4	166.4	124.4
H_2**	97.8	95.7	97.2	100.3	99.6	65.2	97.4	145.1	96.5

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	118.1	88.9	114.8	157.5	116.0	84.4	109.3	155.9	115.3
2	130.1	88.6	105.4	166.5	119.0	----	117.1	169.7	107.6
3	111.1	78.2	101.9	151.2	119.3	81.4	97.0	162.5	121.7
4	108.5	82.0	101.4	144.8	107.5	70.5	98.7	148.3	-----
5	126.0	83.6	126.2	162.3	107.5	77.2	97.8	166.0	119.1
6	119.9	87.1	114.1	152.9	109.5	79.5	106.2	148.7	115.3
7	148.6	96.3	-----	186.7	153.8	96.2	120.8	179.9	147.6
8	112.2	73.1	110.2	150.9	122.5	70.6	99.2	-----	117.4
9	-----	87.4	131.7	178.5	148.5	96.8	150.2	165.5	137.7
10	119.9	80.7	110.6	171.5	111.5	85.2	116.3	161.3	125.7
Mean	121.6	84.0	112.9	162.8	130.9	80.4	109.8	161.9	121.3
H_1*	131.0	89.3	120.9	172.2	150.0	89.8	120.0	169.3	130.4
H_2**	112.2	78.7	105.0	153.5	109.9	75.0	99.6	154.5	116.2

*H_1- 95% confidence interval upper limit
**H_2- 95% confidence interval lower limit

Appendix: Table 5 Raw Data Minute Volume (l/kg/min)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.152	0.153	0.205	0.139	0.142	0.095	0.163	0.205	0.196
2	0.105	-----	0.125	0.133	0.122	0.125	0.068	0.181	-----
3	0.109	0.099	0.088	0.096	0.089	0.085	0.115	0.177	-----
4	-----	0.076	0.092	0.086	0.104	0.133	0.080	0.159	0.103
5	0.334	0.228	0.189	0.175	0.241	0.244	0.218	0.294	0.186
6	0.143	0.119	0.074	0.141	0.105	0.146	0.139	0.166	0.136
7	0.103	0.114	0.117	0.108	0.120	0.061	0.086	-----	0.059
8	0.137	0.103	0.107	0.102	0.171	0.163	0.214	0.246	0.167
9	0.111	0.149	0.095	0.116	0.091	0.137	0.108	0.210	0.152
10	0.105	0.099	0.102	0.132	0.161	0.154	0.125	0.207	0.186
Mean	0.139	0.129	0.129	0.133	0.135	0.134	0.131	0.205	0.145
M_1*	0.172	0.177	0.166	0.158	0.168	0.170	0.169	0.238	0.183
M_2**	0.106	0.099	0.092	0.108	0.101	0.098	0.094	0.172	0.107

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.290	0.232	0.302	0.311	0.307	0.248	0.307	0.400	0.282
2	0.305	0.192	0.286	0.296	0.297	0.231	0.286	0.437	0.300
3	0.276	0.276	0.295	0.269	0.306	0.202	0.286	0.384	0.321
4	-----	0.191	0.262	0.256	0.285	0.202	0.263	0.351	0.286
5	0.301	0.286	0.293	0.295	0.306	0.223	0.274	0.378	0.250
6	0.289	0.281	0.284	0.281	0.289	0.218	0.278	0.375	0.248
7	0.274	0.200	0.220	0.218	0.207	0.157	0.273	0.373	0.239
8	0.196	0.177	0.103	0.215	0.190	0.090	0.195	0.346	0.192
9	0.265	0.257	0.257	0.275	0.266	0.196	0.261	0.401	0.298
10	0.281	0.270	0.256	0.280	0.283	-----	0.269	0.388	0.291
Mean	0.270	0.260	0.266	0.270	0.276	0.196	0.269	0.383	0.271
M_1*	0.298	0.288	0.291	0.292	0.294	0.233	0.290	0.402	0.298
M_2**	0.242	0.232	0.241	0.247	0.248	0.160	0.248	0.365	0.243

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.542	0.508	0.532	0.506	0.502	0.393	0.503	0.[illegible]	0.[illegible]
2	0.507	0.460	0.482	0.500	0.481	0.382	0.517	0.[illegible]	0.[illegible]
3	0.560	0.505	0.504	0.503	0.509	0.404	0.536	0.[illegible]	0.[illegible]
4	0.476	0.497	0.461	0.468	0.510	0.380	0.488	0.[illegible]	0.[illegible]
5	0.510	0.499	0.503	0.543	0.537	0.408	0.534	0.[illegible]	0.[illegible]
6	0.503	0.481	0.458	0.487	0.480	0.353	0.460	0.[illegible]	0.[illegible]
7	0.471	0.494	0.495	0.481	0.486	0.368	0.[illegible]	-----	-----
8	0.521	0.501	0.531	0.480	0.495	0.381	0.[illegible]	0.[illegible]	0.[illegible]
9	0.527	0.517	0.481	0.483	0.469	0.354	0.[illegible]	0.[illegible]	0.[illegible]
10	0.634	0.575	0.568	0.504	0.539	0.401	0.[illegible]	-----	-----
Mean	0.525	0.506	0.518	0.511	0.511	0.389	0.518	0.[illegible]	0.[illegible]
M_1*	0.559	0.538	0.550	0.540	0.537	0.407	0.558	0.[illegible]	0.[illegible]
M_2**	0.490	0.483	0.482	0.483	0.484	0.371	0.[illegible]	0.[illegible]	0.[illegible]

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.271	0.204	0.264	0.351	0.267	0.194	0.[illegible]	0.[illegible]	0.[illegible]
2	0.285	0.194	0.231	0.264	0.260	-----	0.[illegible]	0.[illegible]	0.[illegible]
3	0.255	0.166	0.234	0.247	0.274	0.187	0.[illegible]	0.[illegible]	0.[illegible]
4	0.278	0.210	0.260	0.271	0.276	0.180	0.[illegible]	0.[illegible]	-----
5	0.293	0.194	0.293	0.280	0.250	0.180	0.[illegible]	0.[illegible]	0.[illegible]
6	0.300	0.218	0.286	0.293	0.274	0.199	0.[illegible]	0.[illegible]	0.[illegible]
7	0.362	0.234	-----	0.455	0.375	0.255	0.[illegible]	0.[illegible]	0.[illegible]
8	0.275	0.179	0.270	0.290	0.300	0.173	0.[illegible]	-----	0.[illegible]
9	-----	0.208	0.313	0.404	0.338	0.230	0.[illegible]	0.[illegible]	0.[illegible]
10	0.274	0.184	0.253	0.291	0.255	0.195	0.[illegible]	0.[illegible]	0.[illegible]
Mean	0.288	0.199	0.267	0.286	0.287	0.197	0.[illegible]	0.[illegible]	0.[illegible]
M_1*	0.311	0.215	0.288	0.409	0.316	0.214	0.[illegible]	0.[illegible]	0.[illegible]
M_2**	0.264	0.185	0.246	0.260	0.258	0.180	0.[illegible]	0.[illegible]	0.[illegible]

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 6 Raw Data Ventilatory Rate (cycles/min)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	54	52	54	55	56	54	53	60	54
2	46	48	48	47	45	--	43	66	48
3	49	45	46	46	44	49	--	52	42
4	66	64	67	61	62	64	66	76	58
5	46	45	45	46	45	--	46	55	42
6	56	58	60	60	60	56	59	70	54
7	62	64	61	62	60	58	62	68	58
8	51	48	50	48	46	48	50	60	50
9	56	60	60	60	58	57	60	74	56
10	54	54	58	60	56	53	54	69	50
Mean	54.0	53.8	54.9	54.5	53.2	54.9	54.8	65.0	51.2
M_1*	59.6	59.1	60.2	59.5	58.4	59.2	60.6	70.1	55.4
M_2**	49.4	48.5	49.6	49.5	50.0	50.6	48.9	59.3	47.0

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	69	66	70	71	72	62	70	82	76
2	87	85	86	90	86	74	84	101	91
3	85	82	84	81	82	71	83	96	88
4	74	70	72	78	73	67	76	80	79
5	80	78	82	80	80	68	80	97	79
6	75	69	71	76	75	57	65	89	68
7	66	67	69	69	68	58	64	92	65
8	86	84	83	87	86	74	90	102	84
9	87	87	86	86	87	68	85	101	89
10	80	79	78	80	78	71	78	98	--
Mean	78.9	77.7	78.6	79.8	78.7	67.0	77.5	93.8	79.9
M_1*	84.3	83.4	84.0	84.6	83.4	71.4	83.8	99.4	86.9
M_2**	73.4	71.0	73.2	75.0	74.0	62.6	71.2	88.2	72.9

*M_1 - 95% confidence interval upper limit
**M_2 - 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1128	120	124	132	121	98	124	148	138
2	118	106	110	118	120	96	124	142	118
3	122	112	116	120	120	92	130	148	124
4	134	136	137	136	136	118	134	140	132
5	142	136	142	145	146	125	146	155	150
6	122	120	122	125	126	116	128	136	132
7	103	104	105	104	103	94	102	---	---
8	110	112	114	112	105	86	102	132	114
9	105	104	104	107	102	86	104	119	106
10	124	124	124	125	122	103	114	---	---
Mean	120.8	117.4	119.8	121.4	120.1	101.4	119.8	132.6	126.7
M_1*	129.7	126.0	128.9	130.3	130.2	111.2	130.2	144.2	137.7
M_2**	111.9	108.8	110.7	112.5	110.0	91.6	109.4	130.1	115.5

Cycle 10 ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	76	62	76	90	78	68	88	95	75
2	85	66	76	94	80	64	76	100	87
3	92	78	86	113	98	77	93	129	104
4	81	65	79	96	74	56	77	94	80
5	85	69	84	104	76	64	81	104	86
6	76	58	75	90	72	53	68	88	72
7	100	80	97	114	101	78	94	110	93
8	75	54	77	104	84	56	72	108	78
9	86	70	88	104	90	72	88	102	85
10	80	63	78	106	72	64	80	104	85
Mean	83.6	66.5	81.6	101.5	82.5	64.6	80.2	105.5	84.7
M_1*	89.2	72.3	86.7	107.7	90.0	70.8	86.5	111.6	90.3
M_2**	78.0	60.7	76.5	95.3	75.0	58.4	75.9	95.0	77.6

*M_1 - 95% confidence interval upper limit
**M_2 - 95% confidence interval lower limit

Appendix: Table 7 Raw Data Cardiac Rate (No/min)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	33	32	32	32	32	32	28	45	34
2	32	33	33	33	37	--	35	51	37
3	27	25	25	23	22	22	22	30	21
4	35	33	33	34	33	33	33	49	33
5	31	32	23	32	31	31	--	47	20
6	38	38	39	39	40	37	38	49	36
7	34	36	33	38	35	27	30	34	36
8	32	32	32	36	30	30	30	36	30
9	34	32	31	36	31	29	35	32	32
10	31	31	40	36	37	39	40	56	39
Mean	32.7	32.4	31.9	33.9	32.8	31.1	32.3	42.9	31.8
M_1*	34.8	34.8	35.9	37.1	36.4	35.0	36.6	49.4	36.4
M_2**	30.6	30.0	27.9	30.7	29.2	27.2	28.1	36.4	27.2

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	58	57	59	57	60	46	55	74	48
2	68	62	66	65	66	52	59	79	66
3	54	49	55	58	58	45	60	82	58
4	56	52	54	54	52	46	57	62	56
5	57	57	59	63	64	44	64	88	61
6	56	58	58	57	60	41	60	71	54
7	40	39	36	39	40	28	36	66	48
8	54	52	54	56	57	41	57	72	55
9	68	68	66	66	65	55	67	80	68
10	62	62	63	61	61	50	59	74	--
Mean	57.3	55.6	57.0	57.6	58.3	44.8	57.4	74.8	57.1
M_1*	62.0	61.4	63.2	63.1	63.8	50.1	63.3	80.3	62.5
M_2**	51.6	49.8	50.8	52.1	52.8	39.5	51.5	69.3	51.7

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	92	82	90	84	82	60	90	80	[illegible]
2	93	92	88	92	96	72	90	66	84
3	116	108	108	112	112	80	111	120	[illegible]
4	102	104	108	109	108	89	106	80	[illegible]
5	64	59	64	66	67	56	68	79	[illegible]
6	86	82	87	88	80	63	83	79	[illegible]
7	90	90	92	91	90	78	90	--	--
8	92	92	93	92	88	70	83	104	[illegible]
9	92	92	92	93	88	66	[illegible]	94	[illegible]
10	109	108	106	109	107	50	[illegible]	--	--
Mean	93.6	90.9	92.8	93.6	91.8	69.2	88.3	87.8	[illegible]
M_1*	103.6	101.4	102.1	103.5	101.9	78.5	100.4	100.3	[illegible]
M_2**	83.6	80.4	83.5	83.7	81.7	59.9	76.2	75.3	[illegible]

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	66	51	67	90	68	49	72	88	[illegible]
2	65	47	63	82	63	47	67	84	[illegible]
3	72	50	66	90	66	48	66	81	[illegible]
4	69	45	67	64	66	42	59	80	[illegible]
5	52	38	48	75	60	36	50	68	[illegible]
6	60	35	47	63	61	36	45	67	[illegible]
7	69	48	64	88	67	46	64	92	[illegible]
8	70	50	52	68	66	46	55	[illegible]	[illegible]
9	64	46	62	83	67	54	62	[illegible]	[illegible]
10	48	38	47	58	60	44	46	[illegible]	[illegible]
Mean	63.5	44.8	58.3	76.4	64.4	44.8	58.2	[illegible]	[illegible]
M_1*	69.2	48.9	64.5	84.7	66.6	48.8	64.9	84.4	[illegible]
M_2**	57.8	40.7	52.1	68.1	62.2	40.8	51.5	[illegible]	[illegible]

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 8 Raw Data Ventilatory Stroke Volume (mls)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.18	1.17	1.51	1.00	1.01	0.68	1.22	1.35	1.44
2	0.85	0.91	0.97	1.06	1.01	----	0.59	1.02	1.37
3	0.86	0.85	0.74	0.81	0.78	0.67	----	1.32	0.75
4	0.56	0.48	0.39	0.50	0.59	0.74	0.43	0.75	0.63
5	1.95	1.93	1.60	1.44	2.03	----	1.80	2.03	1.69
6	0.99	0.20	0.48	0.91	0.67	1.01	0.92	0.92	0.97
7	0.65	0.70	0.75	0.68	0.79	0.41	0.54	0.81	0.40
8	0.90	1.51	1.49	1.51	1.34	1.22	1.54	1.48	1.20
9	1.18	0.91	0.57	0.71	0.57	0.38	0.66	1.04	0.99
10	0.67	0.63	0.64	0.76	0.99	1.00	0.80	1.03	1.29
Mean	0.97	0.93	0.97	0.94	0.96	0.83	0.94	1.18	1.07
M_1*	1.24	1.30	1.24	1.17	1.29	1.04	1.31	1.45	1.36
M_2**	0.69	0.66	0.59	0.70	0.65	0.61	0.58	0.90	0.79

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.38	1.41	1.45	1.45	1.41	1.32	1.45	1.61	1.24
2	1.33	1.26	1.26	1.25	1.31	1.19	1.29	1.65	1.25
3	1.52	1.57	1.64	1.55	1.75	1.33	1.61	1.87	1.71
4	----	1.62	1.42	1.28	1.52	1.17	1.35	1.70	1.41
5	1.49	1.46	1.42	1.46	1.52	1.30	1.36	1.55	1.26
6	1.61	1.71	1.65	1.55	1.62	1.60	1.66	1.76	1.53
7	1.42	1.37	1.53	1.33	1.40	1.13	1.48	1.70	1.53
8	1.26	1.17	1.21	1.36	1.22	0.65	1.18	1.79	1.27
9	1.23	1.19	1.21	1.29	1.24	1.16	1.24	1.60	1.35
10	1.56	1.52	1.46	1.56	1.64	----	1.53	1.92	1.64
Mean	1.42	1.43	1.43	1.41	1.46	1.21	1.42	1.72	1.42
M_1*	1.52	1.56	1.54	1.49	1.59	1.40	1.53	1.80	1.54
M_2**	1.32	1.30	1.31	1.32	1.34	1.01	1.30	1.63	1.29

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.99	2.07	2.01	2.02	2.00	1.91	2.04	2.08	1.99
2	1.67	1.67	1.68	1.63	1.54	1.53	1.60	2.01	1.81
3	1.78	1.75	2.02	1.85	1.90	1.67	1.73	2.05	1.92
4	1.59	1.64	1.52	1.55	1.68	1.44	1.64	2.09	1.87
5	1.59	1.63	1.79	1.66	1.63	1.51	1.76	2.05	1.96
6	1.77	1.72	1.61	1.67	1.65	1.31	1.54	2.40	1.50
7	2.03	2.10	2.09	2.05	2.04	1.83	1.89	----	----
8	1.50	1.44	1.50	1.38	1.52	1.45	1.52	1.69	1.46
9	2.13	2.11	1.96	1.91	1.95	1.75	2.18	2.06	1.88
10	1.99	1.81	1.79	1.79	1.72	1.52	2.10	----	----
Mean	1.80	1.79	1.80	1.75	1.76	1.59	1.80	2.19	1.76
M_1*	1.96	1.96	1.95	1.90	1.90	1.73	1.97	2.46	1.93
M_2**	1.65	1.63	1.64	1.60	1.62	1.45	1.63	1.92	1.59

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.55	1.43	1.54	1.69	1.49	1.36	1.57	1.75	1.54
2	1.53	1.34	1.39	1.77	1.49	----	1.48	1.61	1.40
3	1.21	0.93	1.19	1.34	1.22	1.06	1.05	1.31	1.17
4	1.34	1.26	1.28	1.51	1.45	1.25	1.28	1.58	----
5	1.48	1.21	1.50	1.57	1.41	1.31	1.16	1.60	1.38
6	1.56	1.51	1.52	1.70	1.52	1.50	1.56	1.69	1.60
7	1.49	1.20	----	1.65	1.52	1.33	1.35	1.61	1.31
8	1.50	1.35	1.43	1.54	1.46	1.26	1.38	----	1.51
9	----	1.25	1.50	1.77	1.58	1.34	1.57	1.60	1.61
10	1.50	1.28	1.42	1.62	1.55	1.33	1.40	1.59	1.49
Mean	1.46	1.28	1.42	1.61	1.47	1.33	1.38	1.59	1.48
M_1*	1.55	1.39	1.51	1.70	1.54	1.38	1.50	1.68	1.58
M_2**	1.37	1.16	1.33	1.52	1.40	1.19	1.35	1.50	1.37

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 9 Raw Data Cardiac-to-ventilatory Rate Ratio

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.61	0.62	0.59	0.58	0.57	0.59	0.53	0.75	0.63
2	0.70	0.69	0.69	0.70	0.82	----	0.81	0.77	0.77
3	0.55	0.56	0.50	0.50	0.50	0.45	----	0.58	0.50
4	0.53	0.52	0.49	0.56	0.53	0.52	0.50	0.65	0.57
5	0.67	0.71	0.51	0.70	0.69	----	----	0.86	0.48
6	0.68	0.66	0.65	0.65	0.67	0.66	0.64	0.70	0.67
7	0.55	0.56	0.54	0.61	0.58	0.47	0.48	0.50	0.62
8	0.63	0.67	0.64	0.75	0.65	0.63	0.60	0.60	0.60
9	0.61	0.53	0.52	0.60	0.53	0.51	0.58	0.43	0.57
10	0.57	0.57	0.69	0.60	0.66	0.74	0.74	0.81	0.78
Mean	0.61	0.61	0.58	0.63	0.62	0.57	0.61	0.66	0.62
M_1*	0.65	0.66	0.64	0.68	0.69	0.65	0.71	0.76	0.69
M_2**	0.57	0.56	0.53	0.57	0.55	0.49	0.51	0.57	0.55

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.84	0.86	0.84	0.80	0.83	0.74	0.79	0.90	0.63
2	0.78	0.73	0.77	0.81	0.77	0.70	0.70	0.78	0.73
3	0.79	0.79	0.81	0.82	0.81	0.74	0.82	0.95	0.74
4	0.76	0.74	0.75	0.69	0.71	0.69	0.75	0.78	0.71
5	0.71	0.73	0.72	0.79	0.80	0.65	0.80	0.91	0.77
6	0.75	0.84	0.82	0.75	0.80	0.72	0.92	0.80	0.79
7	0.61	0.58	0.52	0.57	0.59	0.48	0.56	0.72	0.74
8	0.63	0.62	0.61	0.64	0.66	0.55	0.63	0.71	0.66
9	0.78	0.78	0.77	0.77	0.75	0.81	0.79	0.79	0.76
10	0.78	0.79	0.81	0.76	0.78	0.70	0.76	0.76	----
Mean	0.74	0.75	0.74	0.74	0.75	0.70	0.75	0.83	0.73
M_1*	0.80	0.81	0.82	0.80	0.81	0.78	0.82	0.89	0.77
M_2**	0.69	0.68	0.67	0.68	0.70	0.61	0.68	0.76	0.69

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18 C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.72	0.75	0.76	0.69	0.64	0.61	0.73	0.58	0.56
2	0.79	0.87	0.82	0.78	0.80	0.75	0.74	0.67	0.66
3	0.89	0.96	0.93	0.93	0.95	0.96	0.85	0.81	0.80
4	0.76	0.77	0.79	0.80	0.79	0.75	0.79	0.57	0.81
5	0.45	0.43	0.45	0.46	0.46	0.45	0.46	0.52	0.45
6	0.71	0.70	0.71	0.69	0.63	0.54	0.64	0.59	0.57
7	0.87	0.87	0.88	0.88	0.86	0.85	0.88	----	----
8	0.82	0.82	0.82	0.82	0.80	0.81	0.86	0.79	0.70
9	0.88	0.89	0.89	0.87	0.86	0.77	0.60	0.79	0.81
10	0.88	0.87	0.86	0.87	0.88	0.49	0.90	----	----
Mean	0.78	0.79	0.79	0.78	0.77	0.70	0.75	0.64	0.68
M_1*	0.87	0.90	0.89	0.88	0.87	0.81	0.86	0.75	0.81
M_2**	0.68	0.69	0.69	0.68	0.66	0.58	0.65	0.53	0.56

Cycle 10 ± 4 C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.00	0.90	1.20	1.10	1.10	0.94	1.30	1.10	0.81
2	0.77	0.71	0.83	0.87	0.79	0.75	0.83	0.84	0.76
3	0.77	0.64	0.77	0.80	0.67	0.62	0.72	0.65	0.81
4	0.85	0.69	0.85	0.67	0.89	0.75	0.77	0.85	0.85
5	0.61	0.55	0.57	0.70	0.79	0.56	0.62	0.60	0.69
6	0.79	0.60	0.59	0.76	0.85	0.68	0.65	0.76	0.81
7	0.69	0.60	0.66	0.77	0.66	0.59	0.68	0.82	0.72
8	1.00	0.93	0.68	0.65	0.79	0.92	0.76	0.48	0.75
9	0.74	0.60	0.71	0.80	0.74	0.66	0.71	0.80	0.76
10	0.73	0.72	0.60	0.55	0.83	0.69	0.56	0.50	0.59
Mean	0.80	0.70	0.75	0.77	0.80	0.72	0.76	0.74	0.76
M_1*	0.89	0.81	0.88	0.88	0.87	0.81	0.91	0.87	0.85
M_2**	0.71	0.60	0.61	0.66	0.73	0.62	0.61	0.61	0.68

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 10 Raw Data P-Q Interval (sec)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.36	0.33	0.34	0.33	0.32	0.32	0.33	0.24	0.28
2	0.39	0.36	0.36	0.35	0.34	----	0.38	0.28	0.34
3	0.39	0.33	0.31	0.31	0.34	----	0.31	0.22	0.32
4	0.35	0.37	0.36	0.36	0.37	0.37	0.36	0.36	0.40
5	0.28	0.30	0.25	0.29	0.30	0.30	----	0.23	0.29
6	0.23	0.28	0.26	0.28	0.29	0.28	0.28	0.21	0.25
7	0.40	0.39	0.39	0.38	0.38	0.38	0.39	0.30	0.37
8	0.29	0.31	0.30	0.31	0.30	0.28	0.28	0.21	0.27
9	0.24	0.26	0.25	0.24	0.22	0.22	0.24	0.16	0.26
10	0.25	0.24	0.26	0.24	0.24	0.26	0.22	0.20	0.28
Mean	0.32	0.32	0.31	0.31	0.31	0.30	0.31	0.24	0.31
M_1*	0.37	0.35	0.35	0.34	0.35	0.35	0.36	0.28	0.34
M_2**	0.27	0.28	0.27	0.27	0.27	0.26	0.26	0.20	0.27

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.18	0.19	0.18	----	0.21	0.20	0.19	0.16	0.19
2	0.15	0.15	0.16	0.18	0.19	0.18	0.17	0.18	0.18
3	0.16	0.17	0.17	0.16	0.18	----	0.17	0.13	0.16
4	0.18	0.19	0.17	0.20	0.17	0.20	0.21	0.16	0.19
5	0.21	0.20	0.18	0.18	0.17	0.21	0.16	0.12	0.17
6	----	----	0.24	0.26	0.24	0.34	0.26	0.24	0.28
7	0.28	0.28	0.29	0.26	0.28	0.38	0.30	0.20	0.28
8	0.21	0.23	0.23	0.20	0.22	0.28	0.23	0.18	0.24
9	0.18	0.18	0.19	0.19	0.19	0.20	0.20	0.16	0.18
10	0.20	0.21	0.21	0.22	0.22	0.26	0.20	0.20	----
Mean	0.19	0.20	0.20	0.21	0.21	0.25	0.21	0.17	0.21
M_1*	0.22	0.23	0.23	0.23	0.23	0.30	0.24	0.20	0.24
M_2**	0.17	0.17	0.17	0.18	0.18	0.20	0.18	0.15	0.17

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.12	0.12	0.11	0.10	0.11	0.12	0.13	0.09	0.11
2	0.16	0.14	0.13	0.14	0.13	0.16	0.11	0.09	0.11
3	0.08	0.09	0.09	0.10	0.09	0.11	0.08	0.06	0.09
4	0.09	0.12	0.11	0.13	0.11	0.11	0.12	0.09	0.11
5	0.11	0.11	0.11	0.10	0.09	0.12	0.10	0.07	0.09
6	0.13	0.15	0.15	0.14	0.13	0.10	0.12	0.10	0.09
7	0.10	0.10	0.11	0.11	0.11	0.13	0.11	----	----
8	0.12	0.11	0.11	0.11	0.12	0.14	0.11	0.11	0.11
9	0.13	0.13	0.12	0.12	0.14	0.19	0.13	0.11	0.14
10	0.09	0.10	0.10	0.11	0.11	0.11	0.11	----	----
Mean	0.11	0.12	0.11	0.12	0.11	0.13	0.11	0.09	0.11
M_1*	0.13	0.13	0.13	0.13	0.13	0.15	0.12	0.10	0.12
M_2**	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.07	0.09

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.18	0.23	0.18	0.13	0.17	0.23	0.16	0.13	0.18
2	0.15	0.22	0.14	0.11	0.14	0.21	0.13	0.09	0.15
3	0.19	0.19	0.21	0.15	0.21	0.30	0.22	0.15	0.22
4	0.24	0.22	0.25	0.17	0.26	0.35	----	0.20	0.25
5	0.20	0.30	0.23	0.16	0.22	0.33	0.23	0.15	0.21
6	----	----	0.27	0.10	0.27	0.42	0.29	0.21	0.27
7	0.19	0.29	0.17	0.14	0.17	0.25	0.16	0.12	0.16
8	0.18	0.23	0.16	0.13	0.17	0.21	0.20	0.11	0.16
9	0.13	0.20	0.15	0.11	0.14	0.16	0.14	0.12	----
10	0.18	0.26	0.19	0.13	0.16	0.24	0.18	0.12	----
Mean	0.18	0.24	0.20	0.13	0.19	0.27	0.24	0.14	0.20
M_1*	0.21	0.27	0.23	0.15	0.22	0.33	0.35	0.17	0.24
M_2**	0.16	0.21	0.16	0.12	0.16	0.21	0.13	0.11	0.16

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 11 Raw Data Q-S Interval (sec)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.08	0.08	0.08	0.07	0.07	0.07	0.06	0.07	0.07
2	0.09	0.08	0.08	0.08	0.07	----	0.08	0.06	0.08
3	0.07	0.06	0.07	0.07	0.07	----	0.07	0.04	0.20
4	0.08	0.09	0.08	0.09	0.08	0.08	0.08	0.05	0.10
5	0.10	0.10	0.09	0.09	----	0.09	----	0.07	0.10
6	0.13	0.11	0.18	0.10	0.06	0.12	0.15	0.10	0.16
7	0.11	0.20	0.16	0.17	0.16	0.18	0.17	0.13	0.16
8	0.17	0.19	0.14	0.13	0.21	0.21	0.20	0.19	0.20
9	0.10	0.06	0.07	0.09	0.11	0.11	0.11	0.10	0.07
10	0.13	0.10	0.12	0.12	0.13	0.10	0.11	0.10	0.09
Mean	0.11	0.11	0.11	0.10	0.11	0.12	0.11	0.13	0.12
M_1*	0.13	0.14	0.14	0.12	0.14	0.16	0.15	0.20	0.16
M_2**	0.09	0.07	0.08	0.08	0.07	0.08	0.08	0.05	0.09

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.07	0.07	0.07	----	0.07	0.07	0.07	0.07	0.07
2	0.07	0.07	0.06	0.06	0.06	0.07	0.06	0.06	0.06
3	0.07	0.07	0.08	0.07	0.06	----	0.07	0.05	0.07
4	0.07	0.06	0.07	0.06	0.05	0.06	0.06	0.05	0.06
5	0.05	0.05	0.05	0.04	0.05	0.07	0.06	0.05	0.08
6	0.08	0.08	0.08	0.07	0.06	0.06	0.06	0.06	0.06
7	0.06	0.06	0.06	0.07	0.06	0.07	0.07	0.06	0.07
8	0.06	0.04	0.06	0.06	0.06	0.06	0.04	0.06	0.06
9	0.10	0.10	0.08	0.08	0.07	0.08	0.07	0.06	0.07
10	0.08	0.07	0.07	0.07	0.07	0.08	0.07	0.06	----
Mean	0.07	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.07
M_1*	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.06	0.07
M_2**	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.06

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.06	0.06	0.06	0.10	0.06	0.08	0.06	0.04	0.08
2	0.05	0.05	0.06	0.05	0.05	0.05	0.06	0.05	0.05
3	0.06	0.06	0.05	0.05	0.05	0.07	0.06	0.05	0.05
4	0.07	0.06	0.06	0.06	0.06	0.08	0.05	0.07	0.06
5	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06
6	0.07	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.05
7	0.07	0.07	0.06	0.06	0.06	0.05	0.06	----	----
8	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06
9	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05	0.05
10	0.05	0.05	0.05	0.06	0.05	0.04	0.04	----	----
Mean	0.06	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.06
M_1*	0.07	0.06	0.06	0.07	0.06	0.07	0.06	0.06	0.07
M_2**	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.06	0.07	0.06	0.05	0.07	0.08	0.07	0.06	0.07
2	0.15	0.26	0.20	0.16	0.21	0.27	0.15	0.14	0.16
3	0.07	0.07	0.07	0.06	0.07	0.08	0.08	0.06	0.08
4	0.08	0.08	0.08	0.07	0.07	0.08	0.07	0.06	0.08
5	0.08	0.08	0.09	0.07	0.08	0.07	0.07	0.06	0.08
6	0.07	0.07	0.08	0.06	0.07	0.07	0.07	0.08	0.07
7	0.09	0.21	0.08	0.11	0.09	0.09	0.09	0.08	0.09
8	0.08	0.09	0.07	0.06	0.08	0.09	0.08	0.07	0.08
9	0.09	0.09	0.09	0.08	0.09	0.10	0.10	0.08	----
10	0.07	0.09	0.08	0.07	0.08	0.08	0.09	0.08	----
Mean	0.08	0.11	0.09	0.08	0.09	0.10	0.09	0.07	0.09
M_1*	0.10	0.15	0.12	0.10	0.12	0.14	0.10	0.09	0.11
M_2**	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.06	0.06

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 12 Raw Data S-T Interval (sec)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.71	0.69	0.70	0.71	0.73	0.70	0.82	0.54	0.69
2	0.58	0.59	0.57	0.58	0.53	----	0.54	0.40	0.48
3	0.74	0.68	0.72	0.74	0.73	----	0.72	0.64	0.86
4	0.65	0.60	0.63	0.61	0.64	0.60	0.62	0.44	0.61
5	0.74	0.72	0.82	0.75	0.75	0.73	----	0.51	0.82
6	0.51	0.51	0.48	0.56	0.56	0.58	0.60	0.47	0.58
7	0.62	0.57	0.62	0.61	0.62	0.61	0.63	0.50	0.67
8	0.69	0.70	0.76	0.63	0.63	0.66	0.64	0.46	0.65
9	0.68	0.66	0.68	0.61	0.69	0.67	0.67	0.49	0.62
10	0.62	0.78	----	0.43	----	0.64	0.63	0.26	----
Mean	0.65	0.65	0.66	0.62	0.65	0.65	0.65	0.47	0.66
M_1*	0.71	0.71	0.74	0.69	0.71	0.69	0.71	0.54	0.75
M_2**	0.60	0.60	0.59	0.56	0.59	0.61	0.59	0.40	0.57

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.36	0.36	0.37	----	0.42	0.48	0.40	0.32	0.43
2	0.35	0.36	0.38	0.38	0.39	0.45	0.36	0.30	0.39
3	0.40	0.38	0.37	0.37	0.38	----	0.37	0.27	0.38
4	0.47	0.45	0.46	0.48	0.46	0.55	0.51	0.43	0.49
5	0.39	0.40	0.42	0.40	0.39	0.53	0.44	0.26	0.41
6	0.35	0.36	0.34	0.37	0.36	0.50	0.38	0.32	0.40
7	----	0.52	----	0.60	0.53	0.78	0.64	0.46	0.64
8	0.40	0.35	0.52	0.48	0.43	0.58	0.46	0.30	0.51
9	0.44	0.45	0.49	0.50	0.53	0.59	0.52	0.32	0.48
10	0.40	0.43	0.43	0.43	0.41	0.46	0.44	0.34	----
Mean	0.40	0.41	0.42	0.45	0.43	0.55	0.45	0.33	0.46
M_1*	0.44	0.45	0.47	0.50	0.47	0.62	0.51	0.38	0.52
M_2**	0.37	0.37	0.37	0.39	0.39	0.47	0.39	0.29	0.40

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.18	0.22	0.25	0.25	0.25	0.40	0.26	0.22	0.29
2	0.30	0.30	0.29	0.27	0.24	0.36	0.25	0.23	0.27
3	0.21	0.22	0.20	0.22	0.24	0.31	0.21	0.21	0.23
4	0.26	0.25	0.26	0.23	0.26	0.30	0.22	0.22	0.25
5	0.35	0.39	0.38	0.37	0.37	0.44	0.31	0.34	0.39
6	0.23	0.28	0.28	0.28	0.29	0.35	0.27	0.15	----
7	0.26	0.26	0.26	0.27	0.26	0.32	0.25	----	----
8	0.23	0.23	0.22	0.23	0.24	0.30	0.21	0.19	0.20
9	0.27	0.27	0.25	0.25	0.27	0.36	0.35	0.24	0.22
10	0.23	0.23	0.23	0.22	0.23	0.38	0.23	----	----
Mean	0.25	0.27	0.26	0.26	0.27	0.35	0.26	0.23	0.26
M_1*	0.29	0.30	0.30	0.29	0.29	0.39	0.29	0.27	0.30
M_2**	0.22	0.23	0.23	0.23	0.24	0.32	0.22	0.18	0.22

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.36	0.46	0.38	0.27	0.39	0.49	0.38	0.28	0.37
2	0.31	0.35	0.27	0.19	0.32	0.34	0.29	0.18	0.30
3	0.39	0.52	0.44	0.31	0.42	0.55	0.45	0.36	0.46
4	0.40	0.58	0.38	0.32	0.46	0.63	----	0.29	----
5	0.45	0.65	0.50	0.37	0.45	0.66	0.56	0.40	0.46
6	----	----	0.39	0.39	0.50	0.73	0.52	0.33	0.44
7	0.28	0.40	0.28	0.25	0.33	0.49	0.34	0.25	0.39
8	0.42	0.56	0.48	0.34	0.42	0.55	0.46	0.31	0.55
9	0.38	0.51	0.39	0.27	0.35	0.41	0.37	0.21	----
10	0.49	0.61	0.40	0.37	0.48	0.54	0.50	0.36	----
Mean	0.35	0.52	0.40	0.31	0.41	0.55	0.43	0.28	0.42
M_1*	0.43	0.59	0.46	0.35	0.45	0.64	0.50	0.34	0.50
M_2**	0.26	0.44	0.34	0.26	0.36	0.46	0.36	0.23	0.35

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 13 Raw Data QRS Voltage (millivolts)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.30	1.10	1.10	1.15	1.15	1.10	1.18	1.35	1.33
2	0.85	0.45	0.50	0.42	0.40	----	0.45	0.50	0.55
3	0.55	0.45	0.55	0.55	0.25	----	0.20	0.25	0.20
4	0.20	0.12	0.12	0.10	0.12	0.10	0.12	0.20	0.11
5	2.03	1.40	1.75	1.25	1.25	1.40	----	1.75	1.68
6	0.07	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07
7	0.16	0.15	0.15	0.15	0.14	0.14	0.12	0.16	0.09
8	0.18	0.15	0.07	0.07	0.14	0.19	0.18	0.18	0.20
9	0.14	0.11	0.06	0.08	0.11	0.15	0.13	0.10	0.13
10	0.35	0.30	0.12	0.09	0.09	0.09	0.10	0.11	0.05
Mean	0.33	0.34	0.39	0.47	0.39	0.35	0.39	0.41	0.36
M_1*	0.48	0.49	0.59	0.69	0.61	0.54	0.55	0.60	0.56
M_2**	0.18	0.19	0.19	0.26	0.16	0.16	0.22	0.22	0.17

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.40	0.35	0.45	0.38	0.40	0.41	0.45	0.40	0.50
2	0.85	0.70	0.68	0.43	0.28	0.60	0.65	0.55	0.50
3	0.38	0.35	0.30	0.28	0.25	0.24	0.28	0.35	0.25
4	1.35	1.40	1.50	0.90	1.10	2.40	0.50	1.10	1.00
5	1.60	1.65	1.60	1.80	1.80	1.80	1.60	1.50	1.70
6	0.58	0.44	0.12	0.04	0.04	0.12	0.06	0.04	0.02
7	1.05	1.15	1.20	1.25	1.10	0.95	1.00	----	----
8	0.20	0.22	0.28	0.18	0.14	0.16	0.16	0.20	0.20
9	0.80	0.80	0.85	0.70	1.10	1.10	1.08	1.00	1.30
10	0.30	0.30	0.30	0.27	0.25	0.30	0.32	----	----
Mean	0.75	0.74	0.73	0.62	0.65	0.81	0.61	0.62	0.69
M_1*	1.09	1.10	1.11	1.02	1.06	1.36	0.95	1.00	1.10
M_2**	0.42	0.37	0.34	0.23	0.23	0.26	0.27	0.25	0.21

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.20	0.20	0.20	----	0.20	0.20	0.22	0.24	0.20
2	0.22	0.20	0.22	0.22	0.18	0.20	0.24	0.55	0.14
3	0.12	0.16	0.20	0.20	0.12	----	0.18	0.16	0.16
4	0.68	0.66	0.66	0.76	0.65	0.60	0.56	0.70	0.60
5	0.24	0.22	0.20	0.20	0.20	0.24	0.24	0.22	0.24
6	0.60	0.50	0.50	0.50	0.40	0.20	0.60	0.30	0.50
7	0.50	0.65	1.00	1.00	1.10	0.90	0.90	1.00	0.85
8	0.12	0.12	0.15	0.16	0.16	0.20	0.22	0.22	0.09
9	0.42	0.47	0.58	0.60	0.62	0.40	0.45	0.46	0.50
10	0.20	0.20	0.22	0.25	0.24	0.20	0.29	0.28	----
Mean	0.53	0.53	0.40	0.39	0.37	0.41	0.28	0.47	0.44
M_1*	1.04	1.04	0.72	0.72	0.69	0.85	0.56	0.89	0.86
M_2**	0.13	0.13	0.08	0.06	0.05	0.00	0.01	0.04	0.02

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.13	0.10	0.09	0.16	0.17	0.18	0.21	0.18	0.15
2	0.70	0.70	0.80	1.10	1.10	1.00	1.10	1.10	1.00
3	0.65	0.68	0.75	0.83	0.80	0.80	1.00	0.90	0.88
4	0.20	0.15	0.14	0.14	0.14	0.16	0.20	0.14	0.14
5	0.40	0.15	0.18	0.50	0.48	0.13	0.19	0.42	0.40
6	0.21	----	0.17	0.19	0.20	0.18	0.20	0.21	0.19
7	0.45	0.44	0.42	0.50	0.38	0.24	0.32	0.31	0.24
8	0.34	0.32	0.32	0.20	0.30	0.35	0.29	0.24	0.29
9	0.25	0.28	0.38	0.34	0.24	0.23	0.26	0.29	----
10	0.59	0.52	0.56	0.58	0.56	0.56	0.56	0.60	----
Mean	0.41	0.37	0.38	0.45	0.43	0.38	0.43	0.43	0.38
M_1*	0.56	0.55	0.56	0.67	0.65	0.60	0.68	0.66	0.51
M_2**	0.25	0.20	0.20	0.20	0.22	0.17	0.19	0.21	0.06

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 14 Raw Data Maximum Buccal Pressure (mmHg)

8°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.20	0.68	0.83	0.71	0.57	0.58	0.48	0.82	0.20
2	0.20	0.77	0.47	0.39	0.34	0.30	0.34	0.61	0.41
3	0.48	0.41	0.54	0.61	0.61	0.50	0.40	1.05	0.75
4	0.61	0.89	0.88	0.51	0.41	0.50	0.60	0.68	0.85
5	0.41	0.54	0.41	0.75	0.68	----	0.40	1.22	1.02
6	0.68	0.80	0.80	0.92	0.92	0.82	0.85	1.02	0.61
7	0.56	0.68	0.80	0.44	1.09	0.88	0.78	0.88	0.82
8	0.85	0.95	0.95	0.78	0.99	0.88	0.95	1.63	1.02
9	0.46	0.35	0.48	0.38	0.63	0.60	0.38	1.26	0.58
10	0.41	0.61	0.75	0.78	0.61	0.72	0.92	0.99	0.68
Mean	0.49	0.60	0.65	0.62	0.69	0.64	0.61	1.02	0.69
M_1*	0.63	0.78	0.85	0.76	0.86	0.79	0.78	1.23	0.88
M_2**	0.34	0.46	0.51	0.47	0.51	0.49	0.44	0.80	0.51

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	2.31	2.45	2.18	2.18	2.99	1.70	1.09	4.08	2.11
2	1.43	1.25	1.50	2.04	1.65	1.50	1.90	3.54	2.45
3	2.72	2.72	2.72	2.31	3.13	1.63	2.45	3.40	1.50
4	4.08	2.18	3.24	3.81	3.67	3.26	3.94	3.54	3.50
5	1.22	0.95	1.36	1.90	1.63	1.09	1.09	1.70	1.09
6	2.72	2.18	2.86	2.53	2.45	1.36	2.04	3.00	2.44
7	2.31	2.28	2.31	2.45	2.31	2.04	2.72	----	----
8	2.31	2.65	2.72	2.31	2.18	1.63	2.90	3.67	2.45
9	1.09	1.19	1.22	1.50	1.90	1.77	1.50	2.04	1.36
10	2.31	2.24	2.31	2.31	2.45	1.53	2.79	----	----
Mean	2.25	2.01	2.24	2.33	2.43	1.73	2.24	3.17	2.17
M_1*	2.88	2.47	2.73	2.76	2.91	2.16	2.88	3.81	2.78
M_2**	1.62	1.55	1.75	1.91	1.96	1.30	1.60	2.53	1.56

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.50	1.70	1.63	1.56	1.47	0.40	1.30	1.70	1.56
2	1.09	1.10	1.22	1.16	1.10	0.70	1.25	1.50	1.00
3	1.90	1.86	1.77	1.77	2.18	0.62	2.92	2.99	2.70
4	0.95	0.76	0.68	0.92	0.68	0.20	1.08	1.63	1.08
5	0.82	1.71	1.43	1.43	1.28	0.76	1.14	1.97	1.09
6	1.70	2.00	1.21	1.20	0.95	----	1.02	2.38	0.92
7	1.06	1.43	1.63	1.36	0.86	0.38	0.79	1.02	0.58
8	0.52	0.58	0.61	0.68	0.54	0.20	0.38	1.22	0.48
9	1.70	1.66	1.02	1.70	1.70	0.75	1.52	2.38	1.75
10	1.02	1.07	1.50	1.63	1.97	0.75	1.27	2.20	----
Mean	1.33	1.39	1.27	1.34	1.27	0.53	1.27	1.90	1.24
M_1*	1.55	1.61	1.56	1.59	1.67	0.71	1.74	2.33	1.76
M_2**	0.91	0.96	0.98	1.09	0.88	0.35	0.79	1.47	0.72

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.16	0.89	0.89	1.70	1.02	0.78	0.92	1.50	0.93
2	0.82	0.36	0.61	0.95	1.16	0.41	0.75	0.75	0.50
3	1.26	0.68	0.78	1.70	1.30	0.40	1.02	1.97	1.12
4	1.36	0.58	0.88	2.04	0.75	0.95	0.86	1.43	1.22
5	0.60	----	0.68	1.77	0.61	0.41	0.65	1.77	0.53
6	----	0.87	0.82	2.04	0.92	0.88	1.09	1.50	0.74
7	1.33	0.44	0.72	0.95	0.61	0.61	0.72	1.02	0.78
8	1.29	0.44	0.99	1.87	0.85	0.56	0.80	1.97	0.80
9	0.75	0.38	0.75	1.88	0.72	0.43	0.80	1.92	----
10	0.68	0.41	0.77	0.32	0.58	0.48	0.63	1.10	----
Mean	1.03	0.56	0.79	1.59	0.85	0.57	0.84	1.51	0.87
M_1*	1.27	0.72	0.87	1.95	1.05	0.75	0.98	1.80	1.05
M_2**	0.79	0.40	0.71	1.03	0.68	0.41	0.70	1.20	0.70

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 15 Raw Data Maximum Buccal Area (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.17	0.77	0.99	0.99	0.76	0.77	0.66	0.95	0.95
2	0.19	0.85	0.90	0.19	0.23	0.23	0.22	0.90	0.45
3	0.75	0.56	0.67	0.84	0.74	0.75	0.66	1.52	0.80
4	0.83	0.99	1.01	0.66	0.50	0.66	0.80	0.86	0.99
5	0.61	0.62	0.55	0.70	0.72	----	0.67	1.02	0.85
6	0.70	0.86	0.75	1.03	0.92	0.81	0.89	1.32	0.63
7	0.67	0.62	0.76	0.45	0.88	0.80	0.66	0.77	0.82
8	0.75	0.76	0.79	0.63	0.67	0.79	0.73	1.29	1.04
9	0.45	0.35	0.54	0.41	0.72	0.61	0.43	1.30	0.65
10	1.00	0.63	0.83	0.92	0.73	0.78	0.86	1.08	0.89
Mean	0.61	0.64	0.71	0.69	0.69	0.69	0.66	1.10	0.81
M_1*	0.80	0.80	0.88	0.88	0.83	0.83	0.80	1.28	0.94
M_2**	0.42	0.48	0.54	0.49	0.55	0.55	0.51	0.93	0.67

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.73	1.50	1.70	1.69	1.62	0.57	1.40	2.32	1.81
2	1.77	1.77	1.95	2.19	2.04	1.01	1.53	3.08	1.85
3	2.13	2.08	2.10	2.11	2.64	1.01	3.23	4.78	3.83
4	1.17	1.03	0.82	1.03	0.78	0.28	1.72	2.21	1.21
5	1.09	1.27	1.61	1.49	1.61	0.93	1.35	2.20	1.54
6	1.76	1.69	1.49	1.29	1.29	----	0.87	2.51	1.22
7	1.32	1.55	1.92	1.58	1.29	0.56	1.06	1.87	0.75
8	0.87	1.00	1.04	1.04	0.82	0.27	0.63	1.80	0.76
9	1.96	2.02	2.05	2.06	2.47	1.16	1.71	3.85	2.31
10	1.67	1.86	2.16	1.67	2.39	0.85	1.76	3.33	----
Mean	1.54	1.56	1.68	1.67	1.70	0.74	1.53	2.80	1.70
M_1*	1.82	1.84	2.01	1.91	2.17	0.99	2.03	3.48	2.43
M_2**	1.25	1.28	1.36	1.39	1.22	0.49	1.01	2.11	0.97

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	2.60	2.18	3.20	3.46	3.70	3.45	3.35	3.07	3.05
2	2.08	1.98	2.37	2.81	2.84	2.89	2.96	5.80	3.5[illegible]
3	4.84	5.90	4.79	3.68	5.58	3.50	3.68	6.71	4.5[illegible]
4	6.68	7.71	4.77	6.38	6.39	5.07	6.99	6.18	5.6[illegible]
5	1.68	1.61	2.08	1.81	2.00	1.48	1.00	1.93	1.25
6	3.70	2.72	3.60	3.13	2.62	1.57	2.15	4.10	3.61
7	5.30	5.23	4.76	4.54	5.65	3.62	5.97	----	----
8	2.93	3.86	4.01	3.10	3.8[illegible]	1.85	2.91	5.36	3.0[illegible]
9	1.37	1.58	1.65	1.91	3.25	2.74	2.41	3.45	2.40
10	3.27	3.04	3.10	3.17	3.28	1.75	4.64	----	----
Mean	3.45	3.58	3.43	3.40	3.71	2.86	3.50	4.57	3.37
M_1*	4.67	5.06	4.26	4.34	4.86	3.76	4.85	5.84	4.47
M_2**	2.22	2.10	2.61	2.46	2.56	1.66	2.15	3.30	2.27

Cycle 10°± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.43	0.98	1.08	1.94	1.07	0.95	1.00	1.85	1.35
2	1.56	0.72	1.46	1.71	1.25	0.41	0.61	1.27	1.41
3	2.26	1.39	1.77	3.84	2.79	0.86	2.10	4.97	2.56
4	1.35	0.84	0.99	2.05	0.75	0.92	1.22	1.75	1.03
5	1.38	----	1.22	2.08	1.37	0.99	1.69	2.30	1.34
6	----	0.73	1.36	3.80	1.76	0.87	1.42	2.84	1.42
7	1.47	0.76	1.39	2.70	1.29	1.18	1.44	1.98	1.49
8	1.89	0.69	1.39	2.49	1.31	0.66	1.05	2.43	1.02
9	1.42	0.70	1.47	1.68	1.50	0.96	1.94	2.82	----
10	1.72	1.20	2.12	2.76	1.45	1.42	1.71	2.72	----
Mean	1.61	0.89	1.42	2.40	1.45	0.91	1.42	2.47	1.43
M_1*	1.84	1.08	1.65	2.96	1.83	1.11	1.75	3.18	1.67
M_2**	1.38	0.70	1.18	1.84	1.06	0.72	1.09	1.76	1.03

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 16 Raw Data: Minimum Buccal Pressure (mmHg)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.14	0.41	0.31	0.29	0.27	0.40	0.27	0.44	0.20
2	0.07	0.14	0.07	0.15	0.07	0.08	0.14	0.40	0.41
3	0.35	0.24	0.41	0.37	0.37	0.35	0.38	0.48	0.24
4	0.27	0.37	0.34	0.24	0.20	0.25	0.28	0.41	0.34
5	0.14	0.15	0.14	0.28	0.27	----	0.14	0.24	0.17
6	0.41	0.48	0.48	0.65	0.51	0.48	0.48	0.75	0.58
7	0.44	0.27	0.48	0.44	0.44	0.27	0.41	0.37	0.27
8	0.27	0.48	0.41	0.41	0.44	0.44	0.48	0.75	0.34
9	0.11	0.14	0.24	0.19	0.22	0.23	0.08	0.68	0.23
10	0.13	0.17	0.27	0.27	0.19	0.27	0.38	0.34	0.21
Mean	0.23	0.29	0.32	0.33	0.30	0.31	0.30	0.49	0.30
M_1*	0.33	0.38	0.41	0.43	0.40	0.40	0.41	0.61	0.39
M_2**	0.14	0.19	0.22	0.22	0.20	0.21	0.20	0.36	0.22

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.44	0.25	0.40	0.34	0.45	0.02	0.38	0.54	0.57
2	0.27	0.30	0.34	0.44	0.30	0.11	0.40	0.50	0.25
3	0.48	0.25	0.20	0.24	0.27	0.10	0.27	0.54	0.48
4	0.16	0.15	0.16	0.14	0.20	0.15	0.43	0.68	0.15
5	0.27	0.27	0.34	0.41	0.27	0.12	0.19	0.68	0.27
6	0.54	0.52	0.39	0.52	0.34	----	0.34	0.68	0.50
7	0.34	0.41	0.20	0.52	0.48	0.20	0.41	0.52	0.18
8	0.07	0.14	0.15	0.14	0.08	0.07	0.11	0.33	0.14
9	0.34	0.34	0.34	0.26	0.48	0.14	0.35	0.68	0.41
10	0.20	0.24	0.41	0.19	0.48	0.18	0.40	0.84	----
Mean	0.31	0.31	0.35	0.37	0.33	0.12	0.33	0.60	0.33
M_1*	0.42	0.41	0.49	0.43	0.43	0.16	0.40	0.70	0.45
M_2**	0.21	0.20	0.22	0.21	0.23	0.08	0.25	0.50	0.20

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.09	1.36	0.54	0.68	1.77	0.20	0.27	2.99	0.48
2	0.44	0.68	0.68	0.95	0.95	0.54	1.22	1.91	1.09
3	1.22	0.95	1.36	1.09	1.77	0.68	1.09	1.50	0.95
4	3.81	1.63	3.54	3.13	2.99	2.45	2.72	3.47	3.13
5	1.09	0.82	1.36	2.31	2.31	0.54	1.77	2.72	2.31
6	1.90	1.50	1.90	1.63	2.04	1.09	1.90	2.99	2.72
7	1.63	1.63	1.90	1.36	2.04	0.95	1.77	----	----
8	1.63	1.90	2.04	1.77	1.50	0.54	2.58	2.31	2.31
9	0.75	0.88	0.88	0.95	1.16	0.82	1.43	1.77	1.36
10	2.72	2.18	2.86	2.04	1.50	1.36	2.31	----	----
Mean	1.63	1.35	1.71	1.59	1.80	0.90	1.71	2.34	1.91
M_1*	2.34	1.71	2.39	2.13	2.20	1.37	2.04	3.01	2.31
M_2**	0.91	0.99	1.02	1.05	1.39	0.47	1.18	1.77	0.91

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.34	0.20	0.20	0.55	0.41	0.24	0.27	0.54	0.30
2	0.22	0.07	0.31	0.34	0.20	0.10	0.27	0.31	0.13
3	0.41	0.15	0.20	0.68	0.35	0.07	0.20	1.36	0.34
4	1.36	0.18	0.07	1.43	0.34	0.27	1.00	1.22	1.13
5	0.30	----	0.31	1.36	0.34	0.07	0.21	0.85	0.15
6	----	0.32	0.51	2.11	0.65	0.41	0.95	0.95	0.41
7	0.78	0.24	0.41	0.95	0.89	0.34	0.44	0.95	0.51
8	0.38	0.09	0.30	1.87	0.27	0.13	0.34	1.91	0.61
9	0.41	0.19	0.38	0.72	0.38	0.14	0.51	1.43	----
10	0.27	0.07	0.20	0.34	0.21	0.04	0.10	0.79	----
Mean	0.50	0.17	0.38	1.00	0.41	0.18	0.40	0.99	0.43
M_1*	0.77	0.23	0.56	1.81	0.56	0.27	0.65	1.33	0.77
M_2**	0.22	0.10	0.21	0.62	0.25	0.09	0.18	0.65	0.21

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 17 Raw Data Minimum Buccal Area (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.09	0.55	0.51	0.48	0.43	0.56	0.36	0.68	0.24
2	0.05	0.11	0.10	0.35	0.11	0.10	0.14	0.74	0.34
3	0.75	0.34	0.64	0.63	0.62	0.60	0.74	0.52	0.35
4	0.64	0.76	0.70	0.38	0.27	0.40	0.65	0.76	0.99
5	0.23	0.13	0.20	0.29	0.35	----	0.18	0.30	0.34
6	0.64	0.66	0.66	0.72	0.56	0.48	0.54	0.87	0.58
7	0.50	0.39	0.67	0.23	0.41	0.40	0.43	0.50	0.43
8	0.23	0.70	0.51	0.50	0.50	0.57	0.73	1.22	0.79
9	0.13	0.21	0.29	0.26	0.24	0.34	0.12	1.11	0.32
10	0.16	0.21	0.29	0.40	0.18	0.31	0.34	0.40	0.33
Mean	0.34	0.41	0.46	0.48	0.37	0.42	0.42	0.71	0.47
M_1*	0.53	0.58	0.61	0.54	0.49	0.54	0.59	0.92	0.64
M_2**	0.15	0.23	0.30	0.31	0.25	0.30	0.25	0.50	0.30

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.02	0.84	0.46	0.55	1.02	0.25	0.12	1.54	0.60
2	0.47	1.02	0.70	1.18	0.96	0.54	1.80	0.97	0.94
3	1.63	1.27	1.31	1.42	2.18	0.52	1.56	1.68	1.06
4	2.04	1.70	2.33	2.01	2.01	1.41	1.60	4.76	1.65
5	1.28	0.86	1.94	1.55	2.14	0.56	0.31	3.53	3.05
6	2.26	1.64	2.48	1.98	2.52	1.55	2.75	3.03	3.61
7	0.84	0.90	0.84	0.80	0.89	0.75	1.14	----	----
8	2.20	2.61	2.58	2.80	2.19	0.77	2.62	3.21	2.49
9	1.40	1.20	1.18	1.07	1.07	1.45	0.15	2.53	1.68
10	3.68	2.96	3.93	2.83	2.52	1.22	2.58	----	----
Mean	1.68	1.50	1.78	1.64	1.84	0.90	1.35	2.74	1.71
M_1*	2.34	2.03	2.55	2.19	2.30	1.23	2.42	3.85	2.79
M_2**	1.03	0.97	1.00	1.09	1.30	0.57	1.26	1.65	1.03

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.62	0.17	0.51	0.46	0.55	0.05	0.64	0.83	0.87
2	0.49	0.57	0.59	0.66	0.46	0.22	0.53	0.79	0.46
3	0.16	0.11	0.11	0.19	0.25	0.06	0.42	0.74	0.43
4	0.19	0.20	0.20	0.10	0.19	0.12	0.56	0.52	1.25
5	0.33	0.26	0.36	0.39	0.23	0.11	0.23	0.65	0.25
6	0.51	0.32	0.34	0.44	0.29	----	0.31	1.21	0.41
7	0.46	0.65	0.92	0.78	0.40	0.11	0.32	0.44	0.56
8	0.11	0.14	0.16	0.32	0.16	0.09	0.12	0.51	0.14
9	0.51	0.49	0.43	0.33	0.75	0.25	0.52	0.96	0.59
10	0.35	0.32	0.35	0.40	0.59	0.15	0.36	0.82	----
Mean	0.35	0.33	0.40	0.41	0.39	0.13	0.40	0.75	0.55
M_1*	0.48	0.46	0.57	0.55	0.53	0.18	0.52	0.91	0.81
M_2**	0.22	0.19	0.23	0.26	0.25	0.08	0.28	0.58	0.29

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.77	0.50	0.67	1.45	0.79	0.45	0.88	1.78	0.93
2	0.30	0.23	0.30	0.34	0.24	0.10	0.43	0.57	0.40
3	1.10	0.44	0.54	1.53	1.30	0.24	0.63	1.82	0.75
4	1.69	0.52	1.19	1.90	0.35	0.39	0.89	1.13	1.00
5	1.09	----	0.79	1.58	0.50	0.15	0.68	1.54	0.42
6	----	0.92	1.52	2.87	1.01	1.35	1.50	3.16	1.29
7	1.08	0.57	1.02	2.17	0.95	0.68	0.92	2.22	1.10
8	0.54	0.11	0.56	1.95	0.55	0.15	0.45	0.19	0.41
9	0.83	0.24	0.78	1.77	0.83	0.27	0.65	1.76	----
10	0.62	0.32	0.76	0.68	0.35	0.10	0.72	1.09	----
Mean	0.89	0.43	0.81	1.62	0.69	0.38	0.75	1.77	0.77
M_1*	1.20	0.61	1.06	2.14	0.94	0.63	1.00	2.27	1.05
M_2**	0.58	0.24	0.56	1.10	0.45	0.12	0.50	1.27	0.49

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 18 Raw Data Maximum Opercular Pressure (mmHg)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.37	0.20	0.20	0.34	0.24	0.20	0.41	0.61	0.44
2	0.20	0.46	0.27	0.41	0.41	----	0.44	0.41	0.27
3	0.52	0.27	0.41	0.41	0.38	0.28	----	0.29	0.27
4	0.25	0.17	0.27	0.20	0.33	0.32	0.25	0.41	0.14
5	0.20	0.27	0.27	0.27	0.27	----	0.27	0.54	0.20
6	0.55	0.61	0.61	0.72	0.68	0.68	0.68	0.78	0.82
7	0.61	0.68	0.61	0.22	0.56	0.61	0.51	0.61	0.68
8	0.61	0.54	0.68	0.48	0.71	0.65	0.68	1.09	0.82
9	0.22	0.34	0.31	0.38	0.46	0.35	0.35	0.88	0.44
10	0.27	0.48	0.54	0.61	0.48	0.51	0.89	0.89	0.58
Mean	0.37	0.40	0.42	0.40	0.45	0.45	0.50	0.65	0.47
M_1*	0.50	0.53	0.54	0.52	0.57	0.60	0.66	0.83	0.65
M_2**	0.24	0.28	0.29	0.29	0.34	0.30	0.33	0.47	0.29

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.68	0.20	0.27	0.20	0.27	0.01	0.10	1.36	0.27
2	0.20	0.20	0.22	0.20	0.20	0.05	0.25	0.42	0.25
3	0.27	0.40	0.38	0.55	0.14	0.02	0.27	0.27	0.14
4	0.27	0.40	0.41	0.27	0.34	0.05	0.37	0.38	0.20
5	0.30	0.27	0.34	0.27	0.34	0.27	0.30	0.33	0.27
6	0.68	0.54	0.52	0.27	0.18	0.07	0.08	0.68	0.35
7	0.26	0.24	0.24	0.31	0.41	0.11	0.22	0.11	0.11
8	0.08	0.11	0.20	0.22	0.14	0.07	0.05	0.08	0.05
9	0.76	0.53	0.41	0.54	0.68	0.24	0.50	1.02	0.50
10	0.34	0.45	0.24	0.82	0.41	0.15	0.30	1.36	----
Mean	0.38	0.35	0.37	0.38	0.31	0.10	0.24	0.60	0.24
M_1*	0.55	0.45	0.47	0.52	0.43	0.17	0.34	0.95	0.34
M_2**	0.22	0.24	0.28	0.23	0.19	0.04	0.14	0.25	0.13

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.80	0.82	0.48	0.68	1.22	0.54	0.65	0.41	0.14
2	0.44	0.41	0.27	0.41	0.54	0.81	0.82	1.63	0.55
3	0.68	0.68	0.82	0.68	0.82	1.63	1.09	0.54	0.68
4	3.54	1.90	1.50	1.36	1.36	1.36	1.09	1.16	1.22
5	0.27	0.27	0.68	0.82	1.09	1.77	0.41	1.70	1.22
6	0.54	0.68	1.09	0.72	0.68	0.48	0.82	1.56	1.22
7	1.29	1.29	1.09	1.22	1.63	0.82	1.72	----	----
8	0.41	0.44	0.68	0.41	0.41	0.13	0.41	0.27	0.54
9	0.68	0.68	0.68	0.75	0.68	1.02	1.22	0.78	1.22
10	0.95	0.92	1.22	0.68	0.54	0.48	1.09	----	----
Mean	0.96	0.81	0.85	0.97	0.90	0.88	1.15	1.06	0.90
M_1*	1.64	1.15	1.11	1.46	1.19	1.38	1.60	1.96	1.78
M_2**	0.28	0.47	0.56	0.48	0.61	0.48	0.66	0.55	0.55

Cycle 10°± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.68	0.55	0.54	0.82	0.61	0.55	0.58	0.85	0.55
2	----	0.31	0.54	0.72	1.29	0.48	0.75	0.61	0.50
3	0.58	0.39	0.17	0.54	0.70	0.10	0.14	0.41	0.22
4	0.51	0.28	0.68	1.29	0.51	0.51	0.70	1.02	0.50
5	0.35	----	0.34	0.34	0.27	0.34	0.41	0.27	0.15
6	----	0.48	0.51	1.29	0.61	0.51	0.75	1.22	0.58
7	0.61	0.27	0.34	0.41	0.41	0.31	0.31	0.41	0.27
8	0.55	0.52	0.52	0.49	0.41	0.20	0.34	0.68	0.39
9	0.42	0.43	0.55	0.41	0.51	0.34	0.75	0.68	----
10	0.31	0.26	0.39	0.44	0.33	0.26	0.38	0.41	----
Mean	0.50	0.37	0.46	0.68	0.57	0.37	0.51	0.66	0.41
M_1*	0.61	0.44	0.56	0.93	0.73	0.47	0.67	0.87	0.53
M_2**	0.39	0.29	0.35	0.42	0.30	0.27	0.35	0.44	0.28

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 19 Raw Data Maximum Opercular Area (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.28	0.16	0.20	0.36	0.21	0.20	0.42	0.63	0.61
2	0.14	0.38	0.24	0.31	0.32	----	0.47	0.45	0.26
3	0.70	0.44	0.41	0.46	0.50	0.45	----	0.39	0.34
4	0.40	0.49	0.39	0.27	0.30	0.30	0.28	0.47	0.17
5	0.15	0.39	0.25	0.37	0.28	----	0.25	0.53	0.29
6	0.41	0.63	0.51	0.68	0.63	0.64	0.65	0.79	0.92
7	0.44	0.45	0.52	0.20	0.48	0.61	0.42	0.61	0.66
8	0.58	0.49	0.63	0.39	0.52	0.54	0.48	1.36	0.68
9	0.21	0.36	0.30	0.32	0.55	0.37	0.39	0.92	0.45
10	0.28	0.46	0.62	0.67	0.46	0.52	0.78	0.85	0.67
Mean	0.36	0.44	0.41	0.40	0.43	0.45	0.46	0.70	0.51
H_1*	0.49	0.54	0.53	0.52	0.52	0.58	0.59	0.91	0.68
H_2**	0.23	0.33	0.29	0.29	0.33	0.32	0.33	0.49	0.33

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.60	0.33	0.33	0.37	0.31	0.02	0.06	1.56	0.42
2	0.36	0.43	0.46	0.39	0.42	0.19	0.49	0.93	0.46
3	0.21	0.34	0.34	0.50	0.14	0.02	0.22	0.26	0.12
4	0.51	0.41	0.42	0.43	0.40	0.06	0.82	0.35	0.34
5	0.27	0.21	0.27	0.27	0.31	0.37	0.31	0.37	0.34
6	0.60	0.56	0.44	0.30	0.24	0.08	0.09	0.71	0.36
7	0.33	0.35	0.36	0.30	0.31	0.11	0.28	0.12	0.12
8	0.20	0.15	0.33	0.23	0.16	0.06	0.08	0.09	0.06
9	0.87	0.71	0.39	0.59	0.81	0.27	0.50	1.14	0.50
10	0.47	0.79	0.92	0.91	0.87	0.40	0.75	1.67	----
Mean	0.44	0.42	0.42	0.43	0.40	0.16	0.34	0.72	0.30
H_1*	0.59	0.57	0.56	0.57	0.58	0.26	0.51	1.14	0.43
H_2**	0.29	0.26	0.29	0.28	0.22	0.06	0.17	0.30	0.18

*H_1- 95% confidence interval upper limit
**H_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.79	1.36	0.85	0.98	2.60	0.56	4.51	0.62	0.57
2	0.59	0.35	0.33	0.67	1.08	1.30	1.69	3.22	0.93
3	0.53	0.90	0.79	0.76	0.96	0.91	1.64	1.18	0.68
4	2.12	1.70	1.56	1.24	1.24	1.14	1.06	1.59	0.97
5	0.47	0.61	1.28	1.31	1.65	3.13	0.58	2.04	1.02
6	0.83	0.82	1.79	1.13	1.15	0.73	1.22	4.94	2.07
7	2.06	2.01	1.66	1.77	2.33	3.31	0.60	----	----
8	0.37	0.63	0.78	0.47	0.47	0.10	0.41	0.26	0.70
9	0.60	0.59	0.57	0.64	1.11	1.42	1.35	1.49	1.07
10	1.26	1.18	1.41	0.98	0.60	0.45	1.16	----	----
Mean	1.06	1.01	1.10	0.99	1.33	1.31	1.62	2.02	1.31
H_1*	1.56	1.40	1.46	1.26	1.81	2.08	2.47	3.57	1.99
H_2**	0.57	0.63	0.74	0.71	0.85	0.55	0.78	0.47	0.64

Cycle 10°C 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.75	0.59	0.58	0.75	0.60	0.56	0.60	0.70	0.70
2	----	0.67	0.71	1.33	1.45	0.67	0.71	1.15	0.88
3	1.90	1.82	0.44	0.70	0.78	0.15	0.11	0.37	0.37
4	0.74	0.40	0.59	1.01	0.43	0.42	0.61	0.88	0.55
5	0.40	----	0.14	0.26	0.34	0.75	0.46	0.26	0.32
6	----	0.38	0.88	1.06	0.94	0.42	0.77	0.87	0.43
7	0.80	0.57	0.65	0.61	0.39	0.74	0.56	0.71	0.52
8	0.78	0.22	0.63	1.32	0.97	0.37	0.45	0.11	0.37
9	0.85	1.09	1.36	0.78	1.46	0.84	0.16	0.81	----
10	1.48	1.51	2.02	1.80	1.61	1.87	1.82	1.55	----
Mean	0.96	0.81	0.80	0.98	0.82	0.69	0.93	0.71	0.60
H_1*	1.37	1.23	1.18	1.30	1.19	1.05	1.41	1.02	0.81
H_2**	0.56	0.38	0.42	0.66	0.45	0.35	0.44	0.40	0.39

*H_1- 95% confidence interval upper limit
**H_2- 95% confidence interval lower limit

Appendix: Table 20 Raw Data Minimum Opercular Pressure (mmHg)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.41	0.41	0.48	0.73	0.58	0.78	0.54	0.44	0.51
2	0.41	0.38	0.34	0.28	0.65	----	0.41	0.34	0.14
3	0.52	0.34	0.44	0.44	0.54	0.35	----	0.22	0.31
4	0.52	0.41	0.44	0.56	0.52	0.45	0.68	0.68	0.20
5	0.27	0.41	0.41	0.37	0.41	----	0.41	0.82	0.34
6	0.55	0.68	0.72	0.35	0.78	0.58	0.68	1.12	0.41
7	0.03	0.54	0.71	0.27	0.85	0.58	0.51	0.68	0.58
8	0.31	0.54	0.48	0.48	0.48	0.65	0.68	1.22	0.72
9	0.19	0.27	0.27	0.48	0.37	0.30	0.27	1.02	0.46
10	0.22	0.24	0.41	0.51	0.41	0.44	0.75	0.68	0.27
Mean	0.37	0.42	0.47	0.50	0.56	0.53	0.52	0.72	0.39
H_1*	0.46	0.52	0.57	0.63	0.68	0.66	0.64	0.96	0.52
H_2**	0.27	0.33	0.37	0.37	0.45	0.39	0.40	0.49	0.27

11°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.48	0.55	0.54	0.61	0.50	0.05	0.20	1.63	0.48
2	0.45	0.45	0.48	0.45	0.42	0.15	0.50	0.68	0.45
3	0.41	0.55	0.48	0.55	0.24	0.10	0.34	0.41	0.24
4	0.41	0.62	0.65	0.51	0.61	0.40	0.68	0.68	0.22
5	0.61	0.52	0.50	0.41	0.41	0.24	0.44	1.09	0.41
6	0.65	0.57	0.52	0.53	0.52	----	0.22	1.77	0.55
7	0.79	0.82	1.02	0.54	0.61	0.16	0.45	0.30	0.24
8	0.27	0.34	0.48	0.30	0.20	0.11	0.14	0.27	0.14
9	0.86	0.68	0.52	0.57	0.88	0.38	0.47	1.23	0.85
10	0.65	0.82	1.03	1.70	0.36	0.50	0.95	3.13	----
Mean	0.52	0.59	0.62	0.57	0.48	0.23	0.44	1.12	0.40
H_1*	0.71	0.79	0.78	0.74	0.62	0.35	0.61	1.75	0.57
H_2**	0.44	0.48	0.47	0.40	0.33	9.11	0.27	0.49	0.23

*H_1- 95% confidence interval upper limit
**H_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.63	0.68	0.54	1.09	0.75	0.14	1.56	0.27	0.20
2	1.70	1.39	1.77	1.63	2.04	1.57	2.72	4.35	2.72
3	1.90	1.36	1.77	1.50	2.31	0.95	1.90	2.99	2.04
4	5.17	5.16	5.17	4.62	5.03	4.22	5.12	5.64	3.86
5	3.94	3.40	5.03	4.76	5.03	1.36	3.40	8.16	6.06
6	2.04	3.81	3.26	1.63	3.67	2.38	3.95	2.18	4.62
7	3.67	3.70	3.67	3.81	4.76	2.99	4.08	----	----
8	0.82	0.71	0.54	0.61	0.27	0.07	0.82	0.20	0.20
9	1.50	1.33	1.36	1.35	1.09	1.09	2.28	3.13	3.72
10	3.40	2.34	3.47	2.31	2.04	1.60	4.42	----	----
Mean	2.58	2.39	2.66	2.33	2.70	1.64	3.03	3.37	2.73
H_1*	3.56	3.49	3.88	3.41	3.90	2.55	4.02	5.61	4.41
H_2**	1.59	1.29	1.44	1.25	1.41	0.73	2.03	1.12	1.05

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.61	0.34	0.42	1.09	0.68	0.38	0.54	1.23	0.42
2	----	0.31	0.82	1.02	0.95	0.34	0.85	1.09	0.83
3	0.53	0.19	0.07	0.41	0.27	0.04	0.10	0.60	0.27
4	1.19	0.43	1.36	2.65	0.88	0.54	1.45	2.45	1.20
5	0.80	----	0.68	1.22	0.82	0.27	0.82	1.36	0.95
6	----	0.50	1.16	2.45	1.22	0.53	1.29	2.45	0.95
7	1.25	0.82	1.36	2.52	1.14	0.89	1.33	2.57	1.50
8	0.49	0.22	0.68	1.09	0.49	0.07	0.41	1.16	0.47
9	1.09	0.75	1.06	1.97	0.95	0.38	0.99	1.74	----
10	0.72	0.11	0.55	0.95	0.42	0.26	0.54	1.16	----
Mean	0.84	0.41	0.82	1.54	0.78	0.32	0.82	1.58	0.84
H_1*	1.09	0.60	1.12	2.10	1.01	0.55	1.15	2.07	1.18
H_2**	0.58	0.22	0.52	0.97	0.56	0.09	0.52	1.09	0.50

*H_1- 95% confidence interval upper limit
**H_2- 95% confidence interval lower limit

Appendix: Table 21 Raw Data Minimum Opercular Area (arbitrary units)

5°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.82	0.71	0.93	0.81	0.71	0.80	0.74	0.88	0.79
2	0.53	0.37	0.33	0.49	0.49	----	0.57	0.51	0.14
3	0.78	0.48	0.67	0.74	0.81	0.50	----	0.45	0.44
4	0.86	0.91	0.93	0.78	0.56	0.66	0.80	1.20	0.36
5	0.40	0.34	0.36	0.38	0.41	----	0.40	0.87	0.38
6	0.47	0.65	0.68	0.75	0.66	0.45	0.57	0.89	0.51
7	0.83	0.69	0.69	0.33	0.71	0.59	0.56	0.69	0.54
8	0.31	0.70	0.51	0.60	0.58	0.74	0.85	1.36	0.85
9	0.31	0.36	0.32	0.65	0.38	0.39	0.35	1.28	0.58
10	0.78	0.19	0.40	0.55	0.35	0.46	0.45	0.49	0.38
Mean	0.68	0.53	0.59	0.61	0.57	0.57	0.55	0.86	0.50
M_1*	0.85	0.70	0.76	0.73	0.68	0.70	0.66	1.10	0.65
M_2**	0.54	0.36	0.42	0.49	0.45	0.45	0.44	0.62	0.35

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.02	0.82	0.56	1.32	1.78	0.33	1.47	0.19	0.86
2	1.48	1.55	1.38	1.77	1.76	0.93	2.52	3.22	2.18
3	2.48	1.53	2.24	2.04	2.86	0.41	2.86	4.02	2.81
4	4.56	4.47	4.66	4.94	5.62	3.97	5.47	6.35	2.99
5	4.57	4.01	5.47	5.58	5.62	1.92	7.62	9.40	7.65
6	2.77	3.40	3.46	2.27	3.86	2.90	4.35	11.70	4.49
7	3.04	3.03	2.98	3.07	3.36	2.35	3.47	----	----
8	0.99	0.90	0.38	0.77	0.32	0.04	0.65	0.18	0.34
9	2.63	2.27	2.18	2.78	2.30	2.08	3.54	4.05	3.36
10	4.07	3.91	4.36	3.75	3.05	1.58	4.14	----	----
Mean	2.76	2.60	2.77	2.83	3.05	1.65	3.61	4.89	3.01
M_1*	3.72	3.56	4.01	3.93	4.25	2.55	5.03	8.31	4.99
M_2**	1.80	1.62	1.53	1.72	1.85	0.76	2.19	1.46	1.03

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.94	0.86	0.85	1.12	0.74	0.08	0.89	2.60	1.00
2	1.34	0.85	0.95	0.84	0.75	0.39	0.91	1.48	0.94
3	0.92	1.07	0.95	1.16	0.52	0.20	0.80	0.72	0.29
4	1.03	0.99	1.09	1.12	0.87	0.57	0.91	0.71	0.32
5	0.76	0.70	0.67	0.76	0.81	0.50	1.00	1.49	0.55
6	0.91	0.72	0.52	0.80	0.75	----	0.44	1.48	0.66
7	1.05	1.17	1.08	0.60	0.59	0.14	0.87	0.54	0.33
8	0.54	0.63	0.89	0.67	0.43	0.23	0.33	0.56	0.22
9	1.54	1.36	1.13	0.86	1.33	0.54	1.01	2.41	1.31
10	0.81	1.05	1.27	1.62	1.68	0.53	1.24	1.93	----
Mean	0.93	0.94	0.94	0.96	0.85	0.35	0.78	1.39	0.62
M_1*	1.12	1.10	1.10	1.17	1.12	0.50	1.01	1.93	0.92
M_2**	0.75	0.78	0.78	0.74	0.57	0.21	0.55	0.85	0.33

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.55	0.74	1.29	3.06	1.86	0.85	1.43	1.67	1.42
2	----	0.56	0.77	1.07	0.36	0.20	0.68	1.02	0.72
3	2.24	0.88	0.18	1.15	0.54	0.13	0.14	1.44	0.36
4	1.96	0.65	1.45	2.27	1.05	0.67	1.59	2.10	1.51
5	0.80	----	0.42	1.73	0.58	0.37	0.96	1.49	0.90
6	----	0.58	0.89	1.98	1.32	0.64	1.19	2.34	1.02
7	2.83	1.05	1.92	3.59	1.53	1.16	1.86	3.55	2.21
8	0.31	0.13	0.18	2.71	1.24	0.07	0.11	2.26	0.92
9	1.61	1.09	1.58	3.07	1.53	0.56	1.26	2.92	----
10	1.81	0.40	1.74	3.75	0.83	0.83	1.90	4.58	----
Mean	1.64	0.68	1.04	2.44	1.08	0.55	1.21	2.34	1.13
M_1*	2.30	0.91	1.50	3.12	1.44	0.80	1.60	3.11	1.61
M_2**	0.98	0.44	0.58	1.76	0.73	0.30	0.83	1.56	0.66

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 22 Raw Data Area Mean Differential Pressure (arbitrary units)

8°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.43	0.49	0.46	0.45	0.44	0.42	0.38	0.95	0.44
2	0.21	0.15	0.20	0.18	0.21	----	0.16	0.39	0.18
3	0.20	0.19	0.21	0.26	0.23	0.20	----	0.81	0.51
4	0.46	0.44	0.64	0.55	0.45	0.44	0.46	0.80	0.39
5	0.48	0.55	0.30	0.51	0.55	----	0.47	0.77	0.44
6	0.35	0.21	0.19	0.37	0.36	0.15	0.25	0.55	0.39
7	0.22	0.22	0.31	0.35	0.35	0.33	0.32	0.42	0.28
8	0.23	0.21	0.16	0.37	0.25	0.40	0.28	0.68	0.46
9	0.33	0.11	0.23	0.42	0.26	0.26	0.31	0.59	0.42
10	0.30	0.20	0.37	0.40	0.43	0.41	0.19	0.33	0.25
Mean	0.32	0.28	0.30	0.39	0.35	0.33	0.31	0.63	0.38
M_1*	0.40	0.39	0.41	0.46	0.43	0.42	0.40	0.78	0.45
M_2**	0.24	0.17	0.20	0.31	0.27	0.23	0.23	0.48	0.30

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	3.30	2.98	2.92	3.25	3.95	1.99	3.79	----	----
2	2.40	2.28	2.48	2.73	2.72	1.97	2.75	4.35	3.56
3	5.53	5.26	5.05	3.68	5.46	1.55	3.54	8.83	5.37
4	3.71	4.26	3.79	4.01	4.47	3.21	5.47	5.97	3.41
5	4.73	4.35	4.40	4.17	4.04	3.33	5.01	6.18	3.63
6	3.35	3.34	3.49	2.83	2.96	2.00	2.67	4.06	2.86
7	5.27	5.40	5.36	5.23	5.17	4.05	5.20	----	----
8	2.46	2.39	2.58	2.52	2.17	1.63	2.52	3.85	2.17
9	2.71	2.62	2.03	2.57	2.38	1.45	2.88	4.38	2.95
10	3.49	3.36	3.27	3.46	3.32	1.53	4.16	----	----
Mean	3.70	3.62	3.54	3.45	3.66	2.27	3.80	5.37	3.42
M_1*	4.50	4.44	4.33	4.06	4.48	2.93	4.60	7.02	4.34
M_2**	2.89	2.81	2.74	2.83	2.85	1.62	3.00	3.73	2.50

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.44	1.54	1.52	1.75	1.68	0.93	1.67	2.53	1.48
2	1.50	1.50	1.61	1.73	1.65	0.92	1.33	2,75	1.66
3	1.51	1.41	1.34	1.38	1.39	0.77	1.63	2.15	1.54
4	1.51	1.32	1.27	1.63	1.63	0.96	1.57	2.07	1.38
5	1.45	1.41	1.40	1.52	1.81	0.89	1.80	2.44	1.41
6	1.40	1.35	1.27	1.47	1.33	----	0.90	2.80	1.36
7	1.32	1.45	1.42	1.40	1.27	0.47	1.30	1.49	0.93
8	1.18	1.36	1.23	1.28	1.12	0.70	1.00	1.73	1.20
9	1.29	1.46	1.52	1.28	1.43	0.88	1.23	2.50	1.59
10	1.74	1.87	1.88	2.05	2.24	0.89	1.67	2.55	----
Mean	1.43	1.47	1.45	1.55	1.55	0.82	1.41	2.30	1.39
M_1*	1.54	1.58	1.59	1.72	1.78	0.94	1.63	2.61	1.56
M_2**	1.32	1.35	1.31	1.37	1.31	0.70	1.19	1.99	1.22

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.64	0.75	0.95	3.38	2.18	0.80	0.99	2.94	1.55
2	----	0.40	1.10	1.86	1.01	0.46	0.92	1.81	0.79
3	1.36	0.64	1.20	2.65	2.01	0.97	1.49	2.71	1.70
4	1.50	0.88	1.22	2.68	1.65	0.90	----	3.05	1.64
5	1.46	----	1.25	2.49	1.04	0.65	1.27	3.00	1.19
6	----	0.94	1.26	1.77	0.92	0.86	0.88	1.81	1.10
7	2.12	0.66	1.41	3.00	2.54	0.86	1.60	3.86	2.44
8	1.00	0.50	0.95	2.44	1.00	0.69	1.39	2.60	1.08
9	1.37	0.94	0.97	2.82	0.85	0.45	1.16	2.73	----
10	1.27	0.76	1.23	2.47	1.53	0.83	1.53	2.06	----
Mean	1.47	0.72	1.15	2.55	1.47	0.75	1.25	2.67	1.43
M_1*	1.74	0.86	1.27	2.90	1.90	0.88	1.46	3.11	1.85
M_2**	1.19	0.57	1.04	2.21	1.04	0.62	1.04	2.20	1.01

*M_1 - 95% confidence interval upper limit
**M_2 - 95% confidence interval lower limit

Appendix: Table 25 Raw Data Opercular Component (a) of MDP (arbitrary units)

5°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.20	0.34	0.86	0.47	0.50	0.35	0.14	0.53	0.32
2	0.20	0.20	0.00	0.03	0.03	----	0.05	0.19	0.07
3	0.13	0.16	0.19	0.26	0.20	0.18	----	1.10	0.78
4	0.19	0.20	0.49	0.26	0.20	0.20	0.20	0.32	0.72
5	0.13	0.25	0.07	0.20	0.23	----	0.18	0.20	0.19
6	0.15	0.20	0.21	0.34	0.22	0.13	0.20	0.32	0.16
7	0.15	0.19	0.22	0.13	0.13	0.18	0.13	0.23	0.00
8	0.10	0.11	0.05	0.15	0.13	0.23	0.35	0.34	0.14
9	0.08	0.15	0.07	0.36	0.12	0.06	0.26	0.37	0.26
10	0.16	0.05	0.11	0.15	0.17	0.15	0.19	0.21	0.05
Mean	0.19	0.18	0.23	0.24	0.19	0.19	0.19	0.38	0.27
M_1*	0.15	0.25	0.41	0.33	0.28	0.26	0.25	0.58	0.46
M_2**	0.25	0.10	0.05	0.14	0.10	0.12	0.12	0.19	0.08

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.36	0.45	0.41	0.64	0.54	0.71	0.81	1.77	0.41
2	0.21	0.25	0.63	0.25	0.25	0.16	0.37	0.66	0.39
3	0.32	0.59	0.54	0.62	0.55	0.07	0.35	0.23	0.11
4	0.34	0.86	0.88	1.03	0.83	0.61	0.36	0.25	0.32
5	0.45	0.38	0.32	0.49	0.51	0.34	0.76	0.75	0.21
6	0.34	0.29	0.16	0.40	0.48	0.00	0.12	0.81	0.34
7	0.41	0.35	0.14	0.03	0.14	0.03	0.52	0.25	0.26
8	0.35	0.46	0.61	0.37	0.31	0.24	0.26	0.14	0.07
9	0.37	0.75	0.46	0.31	0.35	0.18	0.31	0.74	0.52
10	0.47	0.75	0.88	0.67	0.92	0.34	0.81	1.09	----
Mean	0.50	0.50	0.50	0.50	0.49	0.27	0.47	0.67	0.29
M_1*	0.63	0.63	0.69	0.70	0.66	0.44	0.64	1.03	0.40
M_2**	0.37	0.37	0.32	0.30	0.31	0.10	0.29	0.31	0.18

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	2.03	1.90	1.90	2.48	3.00	1.66	3.46	0.00	----
2	0.65	0.11	0.68	0.81	1.08	0.89	1.26	2.58	1.54
3	1.38	0.50	1.04	1.16	1.36	0.23	1.50	3.70	1.69
4	1.34	1.97	3.27	2.15	3.08	1.34	2.88	4.13	1.19
5	3.29	3.15	3.55	3.53	3.48	1.43	4.31	4.66	4.25
6	0.53	1.76	0.99	0.71	1.07	1.37	1.75	1.97	1.36
7	2.54	2.74	2.63	2.77	2.51	1.60	2.62	----	----
8	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	1.84	1.79	1.46	1.69	0.63	0.63	1.44	2.50	1.68
10	0.70	0.79	0.64	0.69	0.85	0.50	1.86	----	----
Mean	1.44	1.53	1.62	1.60	1.71	0.96	2.01	2.44	1.67
M_1*	2.15	2.25	2.47	2.40	2.57	1.38	2.83	3.91	2.85
M_2**	0.72	0.81	0.76	0.80	0.84	0.53	1.19	0.98	0.49

Cycle 10°± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.77	0.27	0.54	1.55	1.12	0.27	0.53	1.41	0.87
2	0.07	0.30	1.02	1.49	0.89	0.54	1.04	1.53	0.73
3	1.31	0.44	----	0.53	0.16	0.17	0.00	0.24	0.00
4	1.64	0.60	1.06	2.28	1.40	0.56	0.98	2.36	1.44
5	0.48	----	0.08	0.38	0.06	0.03	0.06	0.91	0.11
6	----	0.26	0.55	2.16	1.01	0.11	0.12	1.81	0.44
7	1.52	0.55	1.05	1.81	1.55	0.43	0.97	1.85	1.15
8	0.07	0.04	0.03	0.31	0.07	0.00	0.00	0.36	0.18
9	0.85	0.57	0.88	2.56	0.84	0.36	0.82	1.73	----
10	0.36	0.30	0.22	1.26	0.35	0.34	0.73	0.91	----
Mean	0.79	0.37	0.60	1.43	0.75	0.30	0.53	1.31	0.61
M_1*	1.24	0.51	0.93	2.01	1.14	0.44	0.84	1.80	1.04
M_2**	0.33	0.23	0.28	0.85	0.35	0.15	0.21	0.82	0.18

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 24 Raw Data Buccal Component (b) of MDP (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.49	0.35	0.00	0.42	0.13	0.26	0.36	0.51	0.31
2	0.52	0.24	0.07	0.28	0.29	----	0.27	0.24	0.16
3	0.19	0.17	0.03	0.07	0.08	0.08	----	0.11	0.00
4	0.48	0.35	0.15	0.35	0.28	0.25	0.28	0.50	0.00
5	0.40	0.33	0.28	0.35	0.40	----	0.35	0.64	0.33
6	0.29	0.15	0.22	0.24	0.29	0.18	0.22	0.53	0.30
7	0.13	0.07	0.14	0.07	0.22	0.20	0.21	0.27	0.16
8	0.30	0.06	0.23	0.29	0.17	0.26	0.14	0.61	0.36
9	0.25	0.04	0.25	0.07	0.18	0.22	0.07	0.45	0.20
10	0.24	0.17	0.21	0.25	0.26	0.26	0.08	0.25	0.22
Mean	0.37	0.18	0.18	0.27	0.23	0.21	0.22	0.41	0.19
M_1*	0.40	0.25	0.25	0.35	0.30	0.27	0.30	0.54	0.28
M_2**	0.32	0.11	0.11	0.19	0.16	0.16	0.14	0.28	0.10

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.28	1.08	1.01	0.77	1.37	0.33	1.34	1.44	1.05
2	2.54	1.80	2.05	2.14	1.90	1.28	2.05	3.55	2.33
3	4.43	4.95	4.21	2.90	4.78	1.44	2.40	5.53	3.82
4	4.02	4.62	3.79	4.62	4.62	3.20	4.56	4.92	4.09
5	1.44	1.20	0.97	0.92	0.83	1.92	1.17	0.89	0.40
6	2.87	2.06	2.67	2.50	2.20	0.75	1.51	2.89	2.09
7	3.12	3.02	3.10	2.77	3.55	2.44	3.47	----	----
8	3.04	2.99	3.15	3.17	2.45	1.75	3.05	4.91	2.85
9	1.14	1.16	1.07	1.34	2.01	0.83	1.59	2.16	1.41
10	3.04	2.79	2.25	2.77	2.79	1.30	3 08	----	----
Mean	2.69	2.57	2.43	2.39	2.65	1.52	2.42	3.14	2.26
M_1*	3.49	3.56	3.36	3.32	3.59	2.13	3.21	4.71	3.34
M_2**	1.89	1.58	1.59	1.56	1.71	0.92	1.63	1.56	1.17

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.15	1.17	1.16	1.19	1.21	0.71	0.90	0.91	1.37
2	1.19	1.05	1.29	1.48	1.40	0.76	0.96	2.09	1.27
3	0.99	0.95	1.90	0.88	0.85	0.81	1.35	2.10	1.56
4	0.67	0.46	0.39	0.60	0.55	0.55	1.21	1.87	1.06
5	1.00	1.05	1.15	1.25	1.31	0.55	1.03	1.82	1.20
6	1.07	1.05	1.11	1.13	0.88	0.30	0.78	2.18	1.09
7	0.95	1.10	1.42	1.33	1.12	0.44	0.78	1.34	0.63
8	0.75	0.70	0.61	0.70	0.61	0.26	0.54	1.48	0.53
9	0.65	0.86	1.10	1.02	1.16	0.70	0.94	1.98	1.12
10	1.20	1.20	1.20	1.43	1.53	0.57	0.98	1.75	----
Mean	0.96	0.97	1.14	1.10	1.06	0.55	0.95	1.76	1.09
M_1*	1.11	1.15	1.43	1.33	1.30	0.69	1.11	2.04	1.35
M_2**	0.81	0.80	0.84	0.89	0.83	0.40	0.78	1.47	0.84

Cycle 10°± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.05	0.58	0.58	2.08	1.27	0.52	0.61	1.95	1.16
2	1.52	0.17	0.30	0.85	0.34	0.06	0.18	0.82	0.35
3	0.76	0.20	1.37	3.30	2.50	0.80	1.99	4.60	2.03
4	1.22	0.40	0.80	2.04	0.60	0.42	----	1.22	1.10
5	1.84	1.02	1.58	3.90	1.35	0.62	1.45	2.19	1.17
6	----	0.72	0.81	1.28	0.47	0.06	0.76	0.51	0.25
7	1.68	0.21	0.68	1.92	1.65	0.46	0.88	2.98	1.53
8	1.06	0.48	1.02	3.54	1.00	0.86	1.66	3.53	1.09
9	0.63	0.49	0.32	0.97	0.37	0.20	0.38	1.16	----
10	1.02	0.57	1.13	1.31	1.18	0.49	0.81	1.25	----
Mean	1.20	0.48	0.86	2.11	1.12	0.53	0.97	2.02	0.96
M_1*	1.51	0.67	1.16	2.89	1.58	0.72	1.43	2.96	1.46
M_2**	0.88	0.30	0.56	1.33	0.65	0.34	0.50	1.08	0.47

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 25 Raw Data Reversal Component (c) of MDP (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.36	0.11	0.40	0.45	0.19	0.19	0.12	0.10	0.24
2	0.31	0.09	0.08	0.13	0.10	----	0.15	0.04	0.05
3	0.12	0.14	0.00	0.07	0.05	0.06	----	0.39	0.26
4	0.10	0.03	0.00	0.06	0.03	0.01	0.03	0.02	0.33
5	0.05	0.03	0.05	0.06	0.08	----	0.08	0.07	0.08
6	0.29	0.22	0.24	0.31	0.15	0.21	0.17	0.30	0.07
7	0.06	0.15	0.06	0.02	0.00	0.05	0.02	0.02	0.00
8	0.06	0.06	0.11	0.07	0.07	0.10	0.19	0.27	0.04
9	0.00	0.07	0.09	0.01	0.03	0.03	0.02	0.23	0.05
10	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.13	0.00
Mean	0.09	0.09	0.10	0.12	0.07	0.08	0.10	0.16	0.11
M_1*	0.15	0.14	0.19	0.22	0.11	0.15	0.15	0.25	0.20
M_2**	0.03	0.04	0.01	0.01	0.03	0.01	0.05	0.06	0.03

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.05	0.06	0.05	0.03	0.07	0.07	0.05	0.15	0.17
2	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00
3	0.10	0.11	0.12	0.10	0.08	0.11	0.07	0.18	0.13
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.11
5	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
6	0.08	0.08	0.00	0.05	0.03	0.03	0.00	0.18	0.08
7	0.03	0.01	0.14	0.00	0.00	0.00	0.00	0.00	0.00
8	0.10	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.03	0.04	0.04	0.04	0.10	0.02	0.21	0.05
10	0.13	0.08	0.20	0.05	0.21	0.02	0.12	0.29	----
Mean	0.05	0.06	0.06	0.09	0.04	0.03	0.03	0.11	0.06
M_1*	0.09	0.10	0.11	0.16	0.09	0.06	0.06	0.18	0.11
M_2**	0.01	0.01	0.00	0.03	0.00	0.00	0.00	0.03	0.01

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.00	0.00	0.00	0.00	0.44	0.00	0.00	----	----
2	0.22	0.35	0.00	0.22	0.28	0.13	0.56	1.44	0.39
3	0.28	0.21	0.21	0.40	0.68	0.12	0.00	0.39	0.14
4	1.59	2.33	3.56	3.77	3.24	1.39	1.97	3.17	1.39
5	0.00	0.00	0.09	0.27	0.24	0.00	0.44	0.00	1.10
6	0.06	0.28	0.17	0.38	0.32	0.14	0.58	0.07	0.31
7	0.39	0.35	0.35	0.35	0.89	0.00	0.85	----	----
8	0.65	0.62	0.51	0.86	0.39	0.11	0.53	1.06	0.67
9	0.26	0.31	0.14	0.46	0.14	0.11	0.16	0.28	0.07
10	0.25	0.21	0.21	0.00	0.33	0.39	0.78	----	----
Mean	0.37	0.47	0.52	0.59	0.70	0.22	0.59	0.92	0.57
M_1*	0.71	0.95	1.29	1.35	0.51	0.99	1.96	1.96	1.03
M_2**	0.03	0.00	0.00	0.00	0.04	0.00	0.18	0.00	0.10

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.18	0.10	0.15	0.25	0.25	0.09	0.14	0.42	0.48
2	0.28	0.07	0.20	0.48	0.22	0.14	0.30	0.53	0.39
3	0.71	0.00	0.17	1.07	0.65	0.00	0.51	2.13	0.38
4	1.36	0.12	0.64	1.64	0.35	0.08	----	0.53	0.90
5	0.86	----	0.41	1.60	0.37	0.00	0.24	0.10	0.09
6	----	0.04	0.08	0.84	0.50	0.11	0.42	0.51	0.12
7	1.08	0.09	0.32	0.74	0.66	0.05	0.28	0.97	0.25
8	0.13	0.02	0.10	1.41	0.07	0.17	0.27	1.39	0.19
9	0.11	0.12	0.21	0.71	0.33	0.10	0.04	0.17	----
10	0.11	0.11	0.12	0.10	0.00	0.00	0.01	0.10	----
Mean	0.54	0.07	0.24	0.88	0.34	0.07	0.25	0.68	0.34
M_1*	0.90	0.11	0.36	1.27	0.51	0.12	0.37	1.13	0.56
M_2**	0.17	0.04	0.12	0.50	0.18	0.03	0.12	0.22	0.12

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 26 Raw Data Relative Minute Volume (arbitrary units)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	25.8	19.4	27.5	27.0	26.3	25.2	22.8	56.7	26.4
2	12.6	9.0	11.3	10.9	12.9	----	9.6	23.3	11.1
3	17.2	11.4	17.5	15.6	14.1	12.0	----	48.8	30.8
4	27.6	26.4	38.3	32.8	27.0	26.4	27.6	48.0	23.7
5	28.8	33.0	18.0	30.7	33.0	----	28.0	46.2	26.5
6	21.0	12.6	11.4	22.4	21.6	9.0	15.0	33.0	23.4
7	15.2	15.1	18.3	21.1	21.2	19.7	19.0	25.4	16.6
8	15.8	12.6	9.5	22.2	14.7	23.8	17.0	40.8	27.5
9	19.3	6.6	15.8	25.2	15.6	15.6	18.6	35.4	25.2
10	13.2	12.0	19.2	24.0	25.8	24.6	11.4	19.8	15.0
Mean	19.7	16.6	18.0	23.2	21.2	19.5	18.8	37.7	22.6
M_1*	27.9	23.5	24.4	27.9	26.1	25.1	23.8	46.6	27.1
M_2**	14.6	9.9	11.7	18.5	16.4	14.0	13.8	28.9	18.1

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	94.8	92.4	91.2	105.0	100.8	55.6	100.1	151.7	88.9
2	89.9	89.8	96.7	103.7	99.0	55.1	79.8	164.7	99.6
3	91.6	84.6	80.4	80.8	79.2	46.2	97.8	129.0	90.6
4	90.6	79.6	76.4	97.6	82.4	57.4	94.2	124.0	82.7
5	87.0	86.8	85.8	91.4	108.8	53.6	108.0	146.4	84.9
6	84.0	80.7	78.0	88.2	79.5	----	58.1	168.2	81.6
7	78.2	86.8	85.1	84.2	76.2	28.4	78.1	89.2	55.9
8	78.8	81.6	75.8	76.8	67.2	42.0	60.0	103.8	72.0
9	71.5	87.8	91.5	76.7	85.5	52.8	73.8	150.5	95.4
10	104.4	112.2	118.8	108.0	141.2	53.3	103.0	159.7	----
Mean	86.2	88.2	86.8	92.9	93.5	49.4	85.3	138.7	83.5
M_1*	90.6	94.9	95.1	106.2	108.7	56.5	98.1	157.7	93.6
M_2**	78.5	81.5	78.5	81.6	78.4	42.3	72.5	119.7	73.4

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	198.4	180.0	175.4	195.2	237.2	119.6	227.7	299.6	204.8
2	144.0	137.0	148.5	165.0	163.2	118.1	164.9	267.3	213.8
3	331.8	315.8	302.8	290.8	327.6	92.9	212.4	339.4	333.4
4	222.6	255.2	227.4	240.5	268.2	192.6	328.0	358.4	204.7
5	284.0	261.0	264.0	250.4	240.4	200.0	300.8	370.7	217.5
6	200.8	200.6	209.1	170.0	177.6	119.9	160.0	243.9	171.6
7	316.2	324.0	321.3	314.9	310.0	242.9	312.1	-----	-----
8	147.8	143.4	155.0	151.2	130.2	98.0	151.0	231.0	130.0
9	162.8	157.0	121.7	154.3	142.9	87.1	172.6	263.0	177.0
10	209.4	201.6	195.9	207.5	191.1	91.8	249.6	-----	-----
Mean	221.8	217.6	212.1	206.8	219.8	136.3	227.9	319.7	205.2
M_1*	270.1	266.4	259.9	243.8	268.8	175.6	276.0	400.5	251.6
M_2**	173.5	168.8	164.3	169.9	170.8	97.0	179.8	236.8	158.8

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	98.4	45.0	57.1	200.8	130.8	48.0	59.4	176.2	93.0
2	74.4	24.0	65.6	111.2	60.6	28.0	55.4	108.8	47.4
3	81.6	38.4	72.0	159.1	120.6	58.2	89.9	167.3	101.9
4	90.0	52.8	73.2	160.8	99.0	54.0	----	185.0	98.4
5	87.6	----	75.0	149.2	62.4	39.0	76.2	180.0	71.4
6	----	56.4	75.6	106.2	55.2	51.6	52.8	108.0	66.0
7	126.6	39.6	84.5	180.0	150.4	51.5	96.0	231.6	146.4
8	60.0	30.0	57.0	146.4	60.0	41.4	83.4	156.0	64.8
9	82.2	56.4	58.2	169.2	51.0	27.0	69.6	163.7	-----
10	76.2	45.6	73.8	148.2	91.8	49.8	91.8	123.6	-----
Mean	86.3	43.1	69.2	152.3	80.4	44.9	74.9	159.3	86.2
M_1*	100.6	51.8	75.1	174.1	114.3	53.5	87.6	186.5	111.9
M_2**	72.1	34.4	62.5	130.5	62.5	37.2	62.3	137.1	60.4

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 27 Raw Data Gill Resistance

[illegible]°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	4.49	2.24
2	[illegible]	----	1.60	1.35	1.73	----	2.35	2.15	----
3	[illegible]	1.52	[illegible]	[illegible]	2.58	2.35	----	4.58	----
4	----	5.79	8.88	6.40	4.33	3.30	5.75	5.03	3.79
5	[illegible]	[illegible]	1.59	[illegible]	2.08	----	2.16	2.62	2.37
6	[illegible]	1.76	2.57	[illegible]	3.43	1.05	1.80	3.31	2.87
7	[illegible]	1.95	[illegible]	[illegible]	2.92	5.41	3.72	----	4.75
8	[illegible]	[illegible]	[illegible]	[illegible]	1.46	2.45	1.31	2.76	2.75
9	[illegible]	0.74	[illegible]	[illegible]	2.86	1.90	2.87	2.81	2.76
10	[illegible]	2.02	[illegible]	[illegible]	2.67	[illegible]	1.52	1.59	1.34
Mean	[illegible]	[illegible]	[illegible]	[illegible]	2.74	[illegible]	2.65	3.26	2.86
M_1*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3.70	4.17	3.72
M_2**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.59	2.35	2.00

[illegible]°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	[illegible]	5.46	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	----	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----	[illegible]	[illegible]	----
Mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_1*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_2**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	6.08	5.21	5.49	6.18	7.57	[illegible]	[illegible]	[illegible]	[illegible]
2	4.73	4.96	5.14	5.45	5.65	[illegible]	[illegible]	[illegible]	[illegible]
3	9.98	10.42	8.50	6.42	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	7.79	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	6.66	6.94	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	11.19	10.93	10.82	[illegible]	[illegible]	[illegible]	12.04	----	----
8	4.72	4.77	4.86	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
9	5.14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
10	5.50	5.84	5.76	[illegible]	[illegible]	[illegible]	[illegible]	----	----
Mean	7.10	6.91	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_1*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_2**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	6.05	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----	[illegible]	----
5	[illegible]	----	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6	----	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	[illegible]	[illegible]	----	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----	[illegible]
9	----	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----
10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	----
Mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_1*	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M_2**	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix Table 28 Raw Data Coughing Rate (No/min)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0
2	0.8	0.8	1.0	0.9	0.8	0.8	0.7	0.0	1.0
3	1.0	1.0	2.0	2.0	0.8	0.8	0.4	0.4	0.2
4	2.0	2.0	2.0	2.0	2.0	2.0	1.0	0.9	0.4
5	1.8	1.5	1.0	1.4	1.2	1.8	1.5	1.0	1.0
6	6.0	3.4	5.0	4.2	3.0	1.0	3.0	0.2	0.6
7	0.1	0.8	1.2	1.4	0.8	0.1	1.2	0.9	0.2
8	0.2	0.2	0.3	0.5	1.4	1.6	2.0	1.7	1.0
9	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
10	0.3	0.3	0.3	0.2	0.4	0.2	0.2	0.2	0.4
Mean	1.3	1.3	1.4	1.4	1.2	0.9	1.1	0.5	0.6
M_1*	2.6	2.6	2.4	2.2	1.8	1.4	1.7	0.9	0.9
M_2**	4.7	4.7	0.4	0.5	0.5	0.4	0.5	0.1	0.3

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.0	0.0	0.2	0.4	0.3	0.3	0.7	0.8	0.6
2	1.0	1.0	1.0	1.0	0.0	1.0	2.0	5.0	6.0
3	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
4	1.0	0.5	0.5	0.5	1.0	0.0	0.0	1.0	1.0
5	1.0	1.0	1.0	1.0	1.0	1.3	1.0	1.0	1.0
6	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
7	2.3	3.7	4.0	2.0	3.0	2.0	3.0	2.0	0.0
8	0.0	0.8	0.8	1.0	0.6	0.0	0.8	0.4	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	---
Mean	0.5	0.7	0.7	0.6	0.6	0.6	0.9	1.0	1.0
M_1*	1.1	1.6	1.6	1.1	1.3	1.1	1.6	2.1	2.5
M_2**	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.0

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	1.0	1.0	0.5	0.8	4.0	4.0	4.0	1.0	1.0
3	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	---	---
8	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
10	0.4	0.4	0.4	0.8	1.0	0.0	0.8	---	---
Mean	0.3	0.2	0.2	0.5	0.6	0.5	0.6	0.3	0.3
M_1*	0.6	0.5	0.4	0.8	1.5	1.4	1.5	0.6	0.6
M_2**	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	1.0	0.7	0.8	1.3	1.0	0.5	1.0	1.5	1.0
2	2.0	1.0	0.8	2.5	1.2	1.0	1.3	2.5	2.0
3	1.0	1.0	0.8	1.2	0.8	0.8	1.3	1.0	1.0
4	1.7	1.2	1.3	1.7	1.3	0.6	1.3	1.5	1.5
5	2.0	1.0	0.7	0.0	2.0	1.8	0.8	0.0	1.3
6	1.1	1.0	2.7	1.0	2.3	1.0	1.5	0.7	1.3
7	0.7	0.5	0.3	0.6	0.4	0.3	0.3	0.2	0.5
8	1.7	0.5	0.5	0.4	0.7	0.6	0.5	0.2	0.8
9	0.8	0.4	0.5	0.6	0.7	0.3	0.8	0.4	---
10	0.4	0.6	0.6	0.3	0.2	1.0	0.7	1.0	2.8
Mean	1.2	0.8	0.9	1.0	1.1	0.8	0.9	0.9	1.4
M_1*	1.6	1.0	1.4	1.5	1.5	1.1	1.2	1.5	1.9
M_2**	0.8	0.6	0.4	0.4	0.6	0.5	0.7	0.4	0.8

*M_1- 95% confidence interval upper limit
**M_2- 95% confidence interval lower limit

Appendix: Table 29 Raw Data Temperature (°C)

2°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	2.1	2.0	1.9	2.0	2.0	2.0	2.1	5.9	2.1
2	2.0	2.0	2.1	2.1	2.0	2.1	2.0	6.0	2.0
3	2.1	2.1	2.0	2.1	2.1	2.3	2.0	6.0	1.9
4	2.0	2.1	2.0	2.0	1.8	2.1	2.0	6.2	2.0
5	2.0	2.1	2.1	2.2	2.0	2.1	2.0	6.1	2.0
6	2.0	1.9	2.0	2.1	2.1	2.0	2.1	5.9	2.1
7	2.1	2.0	2.0	1.8	2.0	2.1	2.0	6.0	1.9
8	1.9	2.1	2.0	2.1	2.1	2.1	2.0	6.0	2.1
9	2.0	2.0	2.0	2.1	2.0	2.0	2.0	6.1	2.0
10	2.0	1.9	2.0	2.2	2.1	2.2	1.9	6.0	2.0
Mean	2.1	2.0	2.0	2.1	2.0	2.1	2.0	6.0	2.0
H_1*	2.1	2.1	2.1	2.2	2.1	2.2	2.1	6.1	2.1
H_2**	2.0	2.0	2.0	2.0	2.0	2.0	2.0	6.0	2.0

10°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	10.1	10.0	9.9	9.9	10.0	6.1	10.1	14.1	10.1
2	9.8	10.0	10.0	10.1	10.1	6.1	9.9	13.9	9.9
3	10.0	10.2	10.1	10.1	10.1	6.0	10.0	14.0	9.8
4	10.2	10.0	9.9	9.9	10.0	6.0	10.0	14.2	10.1
5	10.1	9.9	10.0	9.9	9.9	5.9	9.9	14.0	10.0
6	9.9	10.0	10.1	10.2	9.9	5.9	9.9	13.9	10.0
7	10.1	10.0	10.0	10.0	9.9	6.1	10.1	14.1	10.0
8	10.0	10.0	10.1	9.9	10.1	5.9	9.9	13.9	9.9
9	10.1	10.1	9.9	10.0	10.0	6.1	10.0	14.0	9.9
10	9.9	10.0	10.1	10.1	9.8	6.0	10.0	14.1	10.1
Mean	10.0	10.0	10.0	10.0	10.0	6.0	10.0	14.0	10.0
H_1*	10.1	10.1	10.1	10.1	10.1	6.1	10.0	14.1	10.1
H_2**	9.9	10.0	9.9	9.9	9.9	5.9	9.9	13.9	9.9

*H_1= 95% confidence interval upper limit
**H_2= 95% confidence interval lower limit

18°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	18.0	18.1	17.9	17.9	17.9	13.9	18.1	21.9	18.0
2	17.9	17.9	18.0	18.1	18.0	14.0	18.0	22.2	17.9
3	18.0	18.0	18.1	17.9	18.0	13.9	17.9	22.1	17.9
4	17.9	18.0	18.0	17.9	17.8	14.1	18.0	21.9	18.2
5	17.8	18.1	17.9	18.1	18.0	14.1	18.0	21.9	17.9
6	18.0	17.9	18.0	18.1	17.9	13.9	17.9	21.9	18.0
7	18.0	18.1	17.8	17.9	18.1	13.9	18.0	22.1	18.1
8	18.1	18.2	18.0	18.0	18.2	14.0	17.8	22.1	17.9
9	18.1	18.0	18.1	17.9	18.0	14.0	17.9	22.0	18.0
10	17.9	17.9	18.0	17.9	18.0	13.9	18.0	22.0	18.0
Mean	18.0	18.0	18.0	18.0	18.0	14.0	18.0	22.0	18.0
H_1*	18.0	18.1	18.0	18.0	18.1	14.0	18.0	22.1	18.1
H_2**	17.9	17.9	17.9	17.9	17.9	14.0	17.9	21.9	17.9

Cycle 10° ± 4°C

Fish No.	Time of sample (hrs.) 0	6	12	18	24	30	36	42	48
1	10.1	6.1	10.0	14.1	10.2	5.9	10.0	13.9	10.0
2	10.0	6.0	9.9	14.0	10.1	6.1	10.1	14.0	10.0
3	9.9	6.0	10.1	14.2	10.1	6.0	9.9	14.0	9.9
4	10.2	5.9	10.0	13.9	10.0	6.1	9.8	13.9	10.1
5	9.9	6.0	10.0	13.9	10.0	5.9	10.0	13.9	9.9
6	10.0	5.9	9.9	14.0	9.9	5.9	10.1	14.1	10.2
7	10.0	5.9	10.0	14.0	10.0	6.0	10.1	14.1	10.1
8	9.8	6.0	10.2	14.1	10.1	6.2	9.9	14.0	10.0
9	10.0	6.1	9.9	13.9	10.0	6.0	9.9	14.0	10.0
10	10.1	6.1	9.9	14.0	10.0	6.0	10.0	14.1	9.9
Mean	10.0	6.0	10.0	14.0	10.0	6.0	10.0	14.0	10.0
H_1*	10.1	6.1	10.1	14.1	10.1	6.1	10.0	14.1	10.1
H_2**	9.9	5.9	9.9	13.9	10.0	5.9	9.9	13.9	9.9

*H_1= 95% confidence interval upper limit
**H_2= 95% confidence interval lower limit

Appendix table 30. Physical characteristics of the rainbow trout used in the study.

Acclimated to 2°C.			Acclimated to 10°C.			Acclimated to 18°C.			Acclimated to cycle 10°C ±4°C.		
Sample No.	Wt.	Lg.	Sample No.	Wt.	Lg.	Sample No.	Wt.	Lg.	Sample No.	Wt.	Lg.
1	396	30.5	1	330	31.0	1	469	33.0	1	435	32.4
2	372	29.0	2	380	32.0	2	389	31.0	2	457	33.6
3	389	28.8	3	468	31.5	3	388	32.0	3	436	31.2
4	326	30.0	4	389	31.2	4	449	30.5	4	390	30.5
5	380	30.0	5	397	31.5	5	443	31.2	5	410	30.8
6	388	27.9	6	419	32.0	6	429	31.0	6	399	31.1
7	392	30.5	7	419	32.5	7	443	32.0	7	430	32.5
8	360	30.1	8	424	34.0	8	322	30.0	8	408	30.3
9	365	30.3	9	404	33.0	9	424	30.5	9	421	31.7
10	345	29.8	10	445	33.5+	10	389	30.5	10	439	32.0
Mean	371	29.9		408	32.2		415	31.2		423	31.6
M_1*	388	30.3		435	32.9		445	31.8		437	32.3
M_2**	355	29.5		381	31.5		384	30.5		408	30.9

M_1* - 95% confidence interval upper limit

M_2**- 95% confidence interval lower limit